

**ASSESSMENT OF DIFFERENT TECHNIQUES IN THE
REMOVAL OF A BIOCERAMIC BASED FILLING
MATERIAL IN LONG OVAL CANALS: A MICRO-CT
STUDY**

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ABSTRACT

Introduction: iRoot SP (Innovative Bioceramik, Vancouver, Canada) was introduced as a calcium silicate-based sealer used for filling of the root canal system. However, there are concerns regarding the removal of this sealer. **Aim:** To evaluate the efficiency of different techniques in removing gutta-percha (GP) and iRoot SP[®] (Innovative Bioceramik, Vancouver, Canada) in long-oval shaped canal using micro-computed tomography (micro-CT). **Materials and methods:** Forty-eight single-rooted mandibular premolars with single straight canals were prepared using ProTaper Next[®] system (Dentsply Maillefer, Switzerland) to size X3 (30/.06) and filled with gutta-percha and iRoot SP[®] (Innovative Bioceramik, Vancouver, Canada) using the hydraulic technique 2 mm short of the working length. The samples were randomly divided into four groups (n=12) in each group according to the root filling removal technique: Group 1 Protaper[®] Universal Retreatment system (Dentsply Maillefer, Switzerland), Group 2 Protaper Universal Retreatment system combined with Xylol, Group 3 Protaper Universal Retreatment system combined with Endo Success[™] Retreatment Kit, ET25 tip (Acteon, England), Group 4 Protaper Universal Retreatment combined with Xylol and Endo Success[™] Retreatment Kit, ET25 tip (Acteon, England). All samples were scanned before and after the removal of the root canal filling material using micro-computed tomography imaging to assess the amount of root filling materials left. **Results:** Results showed no statistically significant difference in the remaining root filling material between the groups ($P>0.05$). The apical thirds showed the highest percentage of the remaining root canal filling material compared to the middle and coronal thirds ($P<0.05$). There was no significant difference between the groups in all thirds of the root ($P>0.05$). Results showed no statistically significant difference in the ability to achieve patency between groups ($P>0.05$). **Conclusion:** No technique proved to be superior to the others in removing bio-ceramic root filling-based material. The apical third retained a significantly

higher percentage of its filling material compared to the middle and coronal third. Bio-ceramic sealers are negotiable in single, straight root canal anatomy.

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ABSTRAK

Pengenalan: iRoot SP (Innovative Bioceramik, Vancouver, Canada) telah diperkenalkan sebagai bahan pemeterai berasaskan kalsium silikat dalam rawatan akar gigi. Walaubagaimanapun, terdapat kebimbangan tentang penggunaan bahan pemeterai ini. **Tujuan:** Untuk mengenalpasti efikasi teknik beralainan dalam mengeluarkan gutta-percha (GP) dan iRoot SP® (Innovative Bioceramik, Vancouver, Canada) daripada sistem akar gigi berbentuk bujur dengan tomografi mikro berkomputer (micro-CT). **Bahan dan kaedah:** Empat puluh lapan gigi geraham kecil rahang bawah disediakan dengan sistem ProTaper Next® (Dentsply Maillefer, Switzerland) sehingga saiz X3 (30/0.6). Selepas itu, tampalan akar dilakukan dengan bahan gutta-percha dan iRoot SP® (Innovative Bioceramik, Vancouver, Canada) pada jarak 2mm lebih pendek dari panjang kerja dengan menggunakan teknik hidraulik. Sampel dibahagikan secara rawak kepada empat kumpulan (n=12) mengikut teknik pengeluaran bahan tampalan akar: kumpulan 1, Protaper® Universal Retreatment system (Dentsply Maillefer, Switzerland), kumpulan 2, Protaper Universal Retreatment system bersama Xylol, kumpulan 3, Protaper Universal Retreatment system bersama Endo Success™ Retreatment Kit, tip ET25 (Acteon, England), kumpulan 4, Protaper Universal Retreatment bersama Xylol dan Endo Success™ Retreatment Kit, tip ET25 (Acteon, England). Semua sampel diimbas sebelum dan selepas pengeluaran bahan tampalan akar menggunakan tomografi mikro berkomputer untuk mengenalpasti jumlah bahan tampalan akar yang ditinggalkan. **Keputusan:** Analisa statistic menunjukkan tiada perbezaan nyata antara semua kumpulan berkenaan jumlah bahan penampal akar yang ditinggalkan ($P>0.05$). Hujung sepertiga pangkal akar menunjukkan peratusan tertinggi peninggalan bahan tampalan akar, diikuti oleh bahagian tengah akar, dan akhirnya bahagian korona sepertiga akar ($P>0.05$). Tiada perbezaan nyata di antara peratusan bahan tampalan akar yang ditinggalkan di bahagian tengah akar dan korona sepertiga akar ($P>0.05$). Tiada perbezaan nyata antara kumpulan

di semua bahagian sepertiga akar ($P>0.05$). Analisa statistic menunjukkan tiada perbezaan nyata dalam keupayaan mendapatkan patensi antara kumpulan ($P>0.05$). Kesimpulan: Tiada satu teknik didapati lebih baik dari yang lain dalam mengeluarkan bahan tampalan akar seramik-bio. Hujung sepertiga pangkal akar menyimpan bahan tampalan akar dalam peratusan yang lebih tinggi berbanding dengan bahagian tengah akar dan korona sepertiga akar. Bahan pemeterai seramik-bio dapat dikelurakan daripada anatomi akar mudah.

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LIST OF SYMBOLS AND ABBREVIATIONS

3D	:	3 dimensional
AAE		American association of endodontics
CBCT	:	Cone beam computed tomography
CM		Confocal microscopy
CSBS	:	Calcium-silicate based sealers
CT	:	Computed tomography
EDS	:	Energy-dispersive spectroscopy
EDTA	:	Ethylenediaminetetraacetic acid
ESEM	:	Environmental scanning electron microscopy
GP	:	Gutta-percha
LM		Light microscope
Micro-CT	:	Micro computed tomography
OM		Optical microscopy
P	:	Patency
SEM	:	Scanning electron microscopy
SM	:	Stereomicroscopy
T	:	Time
WL	:	Working length
XRD	:	X-ray diffraction analysis

CHAPTER 1: INTRODUCTION

1.1 Introduction

Endodontic failure is the persistence of microbial infection in the root canal system and/or the periradicular area (Nair, 2004a). The reasons are various and it can happen to the most skilled dentist at any stage of treatment. It has been reported that endodontic failures occur and the percentages of cases that fail to heal can range from 15 - 22% (Ng et al., 2008). The ability to retreat those cases while being as conservative as possible is of major importance. However, numerous factors can affect the outcome of the retreatment procedure (Sjögren et al., 1990).

The use of rotary nickel-titanium instruments has increased dramatically because of its ability to preserve the anatomy of the canal and reduce the time required to perform root canal treatment (Schäfer & Florek, 2003) and retreatment procedures (Hülsmann & Bluhm, 2004). With the introduction of gutta-percha point matching rotary files geometry, the concept of single cone obturation became a valid treatment modality in many of canal types (Belli et al., 2008; W. Zhang et al., 2009).

Root canal sealers play an important role in root canal treatment as it entombs the remaining microorganisms and fills inaccessible areas of prepared root canals (Ørstavik & Ford, 2008). Nowadays it is well understood that when root canals are filled with solid core material, a cement is required for a fluid tight seal that fills the minor gaps between the core material and the dentinal wall of the canal to prevent leakage (Belli et al., 2008). Since the initial development of sealers in the early twentieth century, the biological and technical importance of sealers has made their chemical and physical properties to be the subject of considerable attention. Root canal sealers are categorized according to their main chemical constituents: zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based sealers.

Bioceramic-based sealers contain alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates. The presence of these elements improves the sealer-to-root dentine bonding because of its similar chemical composition to dentine chemical composition (Ginebra et al., 1997). Some studies reported that these sealers are difficult to remove from the root canal system once they are set (Cherng et al., 2001; Hess et al., 2011). However, the data available on the ability to remove these materials from the root canal system is scarce (Oltra et al., 2017)

Micro-computed tomography (micro-CT) is a method of acquiring 3D images of a given object which has recently been adopted in many experimental studies (Hammad et al., 2008a; Hess et al., 2011). Micro-CT is a highly accurate and nondestructive method for studying the root and canal morphology, instrumentation procedures as well as the evaluation of root canal fillings and its constituents (Karatas & Toy, 2014; Nielsen et al., 1995; Rhodes et al., 1999). One study showed high qualitative and quantitative correlations between the histological and micro-CT examination of root canal fillings (Jung et al., 2005).

1.2 Aim of the study

To evaluate the efficiency of four different techniques in removing gutta-percha (GP) and iRoot SP[®] (Innovative Bioceramics, Vancouver, Canada) in long-oval shaped canal using micro-computed tomography (micro-CT).

1.3 The objectives of the study

The objectives of this study were

- 1- To determine the percentage of remaining root filling material in long oval canals using 4 different techniques rotary files, rotary files and Xylol, rotary files and ultrasonic instrument, rotary files, Xylol and ultrasonic instruments using micro-CT.
- 2- To determine the percentage of remaining filling material in the coronal, middle and apical thirds of the canals using micro-CT.
- 3- To assess the ability to re-establish patency in canals obturated with iRoot SP and GP using rotary files, rotary files and Xylol, rotary files and ultrasonic instrument, rotary files and Xylol and ultrasonic instruments using micro-CT.

CHAPTER 2: LITERATURE REVIEW

2.1 Apical periodontitis

Developments in microbiology have made a dramatic change in the understanding of the aetiology and management of chronic diseases of persistent infections. It has been delineated, unlike classical infections caused by a specific aetiological agent, some diseases are caused by a syndicate of microbial species living harmoniously in a biofilm ecological system (Fux et al., 2003). Apical periodontitis is described as an inflammatory disorder of the periapical tissue as a result of an infection in the root canal system.

2.2 Aetiology

Defects in the dental hard tissues, resulting from caries, treatment procedures or trauma-induced fractures and cracks are the most recognized routes of pulpal infection. The role of bacteria in the development of apical periodontitis has been described by Kakehashi et al. (1965). They exposed the pulps of molar teeth in germ-free rats and conventional rats in a controlled environment. Apical periodontitis did not develop in germ-free rats compared with the control rats in which massive periapical lesions occurred. These findings were further supported by others (Möller, 1981; Sundqvist, 1976). Furthermore, Möller defined the role of anaerobic bacteria in the development of apical periodontitis.

The deceased pulp provides a selective habitat for endodontic microflora (Fabricious, 1982). However, the extent of the inflammation is influenced by microbial interaction, microbial interference with host defence and production of endotoxins and enzymes (Nair, 2004b). Apical periodontitis is the host body defence response to the necrosis of the pulp and microbial inhabitation of the root canal system (Kronfeld, 1955). Despite the host impregnable defence, it cannot get rid of the microbes fortified in the necrotic root canal. An equilibrium is pounded allowing for persistence of the pathogens and limits the

host defence, leading to the formation of various categories of lesions of apical periodontitis (Nair, 1997). Thus, apical periodontitis is not self-limiting or self-healing and intervention should be made to treat it.

2.3 Root canal treatment

2.3.1 Definition

Root canal treatment is defined as the mechanical instrumentation accompanied with chemical debridement followed by filling the root canal system with an inert material, designed to maintain or restore the health of the periradicular tissues (Ng et al., 2008). It offers a variety of treatment options with high success rates (Ng et al., 2008). Teeth with infected root canal spaces require thorough antiseptics of the root canal space and the surrounding dentine. Thus, in infected root canal system, the aim is to eliminate the microbes which have infected root canal space (Hargreaves & Berman, 2015).

2.3.2 Phases of root canal treatment

2.3.2.1 Diagnosis

Before accessing into the pulp chamber or performing any other procedure on the tooth it is essential to obtain a correct diagnosis of the pulp and the peri-radicular tissue. This clinical diagnosis should be established upon the history of the symptoms, presenting symptoms, diagnostic tests and clinical observations. One study showed that the interrelation between the clinical symptom and pulpal histopathology is poor (Dummer et al., 1980). Attempts have been made to accurately diagnose the pulp status based only on clinical signs and symptoms, sensibility tests and radiography have not been successful (Eriksen & Dimitrov, 2003). Hence, symptoms and available clinical tests give a poor indication of the pulp and periapical status, as much data as possible should be gathered prior to the formulation of the final diagnosis.

2.3.2.2 Preparatory phase

The aims of the preparatory phase are to remove the infected soft and hard tissue, enable the access of irrigants to apical root canal space, create space for delivery of medicaments and subsequent root filling material and retain the integrity of the radicular tissue (Hargreaves & Berman, 2015). Three main objectives should be met to achieve these goals:

(a) ***Mechanical objective***

The ideal mechanical objective of root canal instrumentation is to achieve complete and centred consolidation of the root canal space into the desired shape. Unfortunately, this goal is unlikely to be achieved with the available technology due to the complex anatomy of the root canal system (Paqué et al., 2009; Peters et al., 2001).

(b) ***Biologic objective***

Proper shaping is essential for proper disinfection of the root canal as it creates a space for delivery of antimicrobial irrigants. Despite the finer needle sizes used nowadays a thorough cleaning of the most apical part is still a challenge due to the complex anatomy of the root canal system (Amato et al., 2011).

(c) ***Technical objective***

Despite the fact that a tapered canal preparation incorporating the original shape and curvature of the canal is a valid goal, an optimal final apical size and the degree of taper are still debatable. While some studies suggested the use of larger apical and less taper for better disinfection (Card et al., 2002), others found no significant difference whether the apical size is small or large (Coldero et al., 2002).

2.3.2.3 Root canal filling phase

After removal of the pulp tissue and the root canal system is properly shaped and disinfected, the wound at the apex must be protected from any external insults. Furthermore, the root canal space must be properly sealed to protect the underlying tissues from any invasion from the bacteria inhabiting the oral cavity. The aim of root canal filling is to provide a three-dimensional seal to prevent reinfection of the root canal system (Schilder, 1967). Many materials have been used to fill the root canal space. Most of which use a core material adjunct with sealer. The American Association of Endodontics (AAE) “guide to clinical endodontics” states, “Root canal sealers are used in conjunction with a biologically acceptable semi-solid or solid obturating material to establish an adequate seal of the root canal system.” (AAE, 2016)

2.4 Root canal filling procedure

There are two primary methods to fill the root canal system. The root canal system can be filled with a solid material shaped to fit the root canal space. Then, it is cemented into the root canal space using a sealer/cement. The second method uses a mucilage material that will harden to the shape of the root canal space or a plasticized material that will set after placement (Ørstavik & Pitt Ford, 2008). Obturation techniques can also be classified according to the mode of compaction and/or the temperature of the filling material. For example, warm vertical and cold lateral (Ingle, 1994).

2.4.1 Methods of root canal filling

2.4.1.1 Lateral compaction

It is the most commonly learnt and practised obturation technique among dentists worldwide (Johnson, 2006; Karr et al., 2007). This technique can be used in almost any clinical situations. Furthermore, it produces a predictable length control of the root filling (Gilhooly et al., 2001). A disadvantage of this technique is that it does not produce a

homologous block. The master cone and the accessory cones remain separate. It is conceptualized that all the spaces are filled by the sealer to produce a water-tight seal (Hargreaves & Berman, 2015). Furthermore, this technique may not be able to completely fill complex root canal systems (Wu & Wesselink, 2001).

2.4.1.2 Warm vertical compaction

Schilder was ahead of his time when he introduced the warm vertical compaction of gutta-percha in an attempt to achieve three-dimensional obturation of the root canal system (Schilder, 1967). As studies showed that bacteria in prepared, unfilled canals can multiply and reach the pre-treatment levels within 4 days (Byström & Sundqvist, 1981). This technique uses a heated spreader in order to remove and pack the gutta-percha. The advantages of using warm vertical compaction lie in its ability to create a homogenous mass of gutta-percha, and filling irregularities and accessory canals in a better manner than lateral compaction (DuLac et al., 1999; Wu et al., 2001). However, the use of flame heated carries produces more than 10°C increase in external root surface temperature which can cause irreversible damage (Lee et al., 1998). Furthermore, because of the compaction forces, length control is poor compared to lateral compaction and there is a higher chance for extrusion of materials into the peri-radicular tissues. Because warm vertical compaction uses stiff pluggers it can be difficult to use in a curved canal where pluggers cannot reach the desired length (Hargreaves & Berman, 2015).

2.4.1.3 Continuous wave compaction

This technique is a variation of the warm vertical compaction technique (Buchanan, 1998). It utilizes an electric heat source instead of using flame. The electric heat source will not only allow for better temperature control but also different temperature settings. External root surface temperature increase using continuous wave compaction was less than 10°C (Lee et al., 1998; Silver et al., 1999).

2.4.1.4 Single cone technique

This technique consists of a single master gutta-percha cone in conjugation with sealer layer thicknesses that vary depending on the fit of the single cone to the walls of the canal (Wu et al., 2009). The absence of a non-filled spreader track (Souza et al., 2009), and the reduced risk of damaging dentine are the major advantages of this technique (Shemesh et al., 2009). As the volume of sealer required in this technique is higher than other techniques a dimensionally stable sealer is required (Wu et al., 2009). The quality of obturation using single cone technique was shown to be as efficient as lateral condensation and continuous wave compaction techniques (Hörsted-Bindslev et al., 2007; Iglecias et al., 2017)

2.4.2 Root canal filling materials

The hunt for the ideal root canal filling material started 200 B.C when they used bronze wire to fill the root canal system. Since then many materials have been introduced for obturation of the root canal system. Ideal properties of root canal filling material have been proposed by Grossman (1958) (

Table 2.1). Most techniques consist of a core material and a sealer. It is worth mentioning that regardless of the core material used, a sealer is of vital importance to achieve a fluid-tight seal. According to Sundqvist and Figdor (1998), the root canal filling material has three primary functions. 1) To seal the canal against microleakage of bacteria from the oral cavity, 2) To entomb the bacteria survived shaping and cleaning procedures, and 3) To engross the root canal system to prevent fluid accumulation which may serve as a nutrient source for bacteria (Sundqvist & Figdor, 1998).

Table 2.1 Grossman criteria for ideal root canal filling material

Ideal properties of a root filling material

- It should be easily introduced into the canal
- It should seal the canal laterally as well as apically
- It should not shrink after being inserted
- It should be impervious to moisture
- It should be bacteriostatic or at least not encourage bacterial growth
- It should be radiopaque
- It should not stain tooth structure
- It should not irritate periapical tissue
- It should be sterile, or quickly and easily sterilized before insertion
- It should be easily removed from the root canal if necessary

2.4.2.1 Core materials

Gutta-percha was introduced by Dr William Montogmerie in 1843 (Prakash et al., 2005). However, it was not used as root canal filling until 1848 when Hill developed the first gutta-percha root filling material known as “Hill’s stopping” (Thorpe, 1909). Gutta-percha is trans-isomer of isoprene compared to the natural rubber which is the cis isomer of isoprene. The structural difference grants gutta-percha some of natural rubber physical characteristics while allowing it to have a crystalline polymer behaviour (Prakash et al., 2005). Crystalline gutta-percha has two forms α and β (Schilder et al., 1974). At room temperature gutta-percha is in β phase. In this state, it is non-sticky, firm, compactible and becomes friable with age. Upon heating 42°-49° C gutta-percha transforms to the α phase. In this phase, it becomes sticky, runny and non-compactible. After cooling gutta-percha transforms back to the β phase. However, it undergoes more shrinkage with cooling than expansion with heating. Therefore, gutta-percha should be compacted to avoid the shortcoming of this problem (Prakash et al., 2005). When the α phase is heated above 69°C it melts. Extremely slow cooling will allow the melted gutta-percha to be recrystallized in the α phase. When the α phase is heated and cooled in undergo less shrinkage which makes it more stable for thermoplasticized techniques (Hargreaves & Berman, 2015).

Gutta-percha is a material with minimal toxicity, minimal irritability and with the slenderest chance of getting an allergic reaction when confined to the root canal space (Nguyen, 1994). Commercially available gutta-percha cones consist of 20% gutta-percha, 65% zinc-oxide, 10% radiopacifiers, and 5% plasticizers (Friedman et al., 1977). Gutta-percha is compressible, inert, dimensionally stable, and well tolerated by tissues. Furthermore, it is radiopaque and can easily be removed with heat or solvents. On the other hand, it lacks in rigidity and length control, it does not adhere to the dentinal walls and can be easily displaced (Prakash et al., 2005). Manufacturers attempted to make gutta-percha antimicrobial by adding materials such as calcium hydroxide (Lohbauer et al., 2005), iodoform (Chogle et al., 2005), and chlorohexidine (Lui et al., 2004). Unfortunately, clinically no additional benefit of using these materials was verified.

2.4.2.2 Root canal sealers

(a) *Zinc-oxide eugenol*

Zinc-oxide eugenol sealers have a long clinical history. It was introduced by Rickert and Dixon 1933 (Hargreaves & Berman, 2015). Although this sealer bears the advantage of having an anti-microbial activity (Heling & Chandler, 1996), and the ability to resorb if extruded beyond the apical end of the canal (Augsburger & Peters, 1990), it has a slow setting time (Allan et al., 2001), it shrinks on setting (Kazemi et al., 1993), and it may stain the teeth if not properly cleaned (Krstl et al., 2013). Furthermore, zinc-oxide eugenol sealers have the disadvantage of being a soluble material (Peters et al., 2002). A non-staining staining version was introduced later by Grossman in 1955 (Grossman, 1958). To avoid the irritating effect of eugenol, a non-eugenol formula was introduced (Hargreaves & Berman, 2015).

(b) ***Calcium hydroxide sealers***

These sealers were developed with the objective of having antimicrobial activity in conjugation with osteogenic-cementogenic potential. Unfortunately, studies established the absence of such actions (Desai & Chandler, 2009). In order for calcium hydroxide to preserve its activity, it has to be soluble (Desai & Chandler, 2009; Mohammadi & Dummer, 2011). This is contradictory with the objective of sealers (Hargreaves & Berman, 2015).

(c) ***Glass-ionomer sealers***

It was logical to introduce a sealer based on glass-ionomer because of its ability to bind to dentine. It was demonstrated that glass-ionomer sealers have the ability to bind to the canal walls (Friedman et al., 1995). However, it is challenging to treat the dentinal walls to receive the cement, especially in the middle and apical thirds. Furthermore, leakage studies showed no statistical difference between glass-ionomer and the more commonly used resin sealers (Fransen et al., 2008).

(d) ***Resin sealers***

Resin sealers have a long clinical history and it has the benefit of adhesion to the dentinal walls. It has two major categories:

The first category is epoxy resin sealers; an example of this category is AH plus. The second is Methacrylate resin sealer, it has four generations up to date. Examples of this category are hydron, EndoREZ and Real Seal (Hargreaves & Berman, 2015).

Resin sealer shows varying degree of cytotoxicity (Eldeniz et al., 2007; Martinho et al., 2018). Some studies reported that resin sealers have good sealing ability (De Moor & De Bruyne, 2004) while other studies demonstrated that resin sealer undergoes shrinkage

upon setting which can compromise the outcome of the endodontic treatment (Hammad et al., 2008b; Ørstavik et al., 2001).

(e) ***Silicone sealers***

On the contrary of resin sealers, silicon sealer expands on setting (Ørstavik et al., 2001). It is biocompatible (Bouillaguet et al., 2006) and showed consistent results in filling canals irregularities (Zielinski et al., 2008). On the other hand, it has inconsistent setting time and it might be affected by the last rinse of sodium hypochlorite (Bouillaguet et al., 2006). Furthermore, some studies showed good sealing ability compared to other sealers while others showed inferior results (Hargreaves & Berman, 2015).

(f) ***Bioceramic sealer***

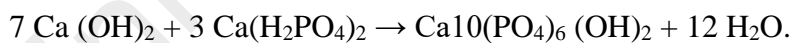
Bioceramic is a term introduced for material that contains calcium silicate. They can be classified as bioinert, bioactive or biodegradable according to the interaction with the surrounding tissues (Raghavendra et.al 2017). Bioceramics does not induce a foreign body reaction (de Oliveira et.al 2018). It is mainly composed of alumina and zirconia, bioactive glass, glass ceramics, calcium silicate, hydroxyapatite and resorbable calcium phosphate, and radiotherapy glass (Best et.al 2008). As a main ingredient of bioceramics, calcium-silicate belongs to bioactive materials and can induce growth of the surrounding tissues (Raghavendra et.al 2017). Cements based on a composition of calcium and silicate such as mineral trioxide aggregate (example, ProRoot MTA, Dentsply Sirona, York, USA) or Biodentine (Septodont, St.Maur-des-Fosses, France) have been recently introduced to the dental practice in past two decades and endodontology was no exception. These cements have a wide range of applications clinically such as pulp capping in both primary and permanent teeth, retrograde filling and perforation repair.

The first sealer of the new genera was introduced in 2007 was branded as iRoot SP (Innovative Bioceramix, Vancouver, Canada). Bioceramic sealers achieved a biological

point of view on the obturation of root canal systems because of their excellent sealing ability and biocompatibility. Hence, the fluid-tight seal is no longer the only objective of root canal obturation. Bioactive inducement of healing of periapical tissue, hard tissue formation and anti-bacterial properties are added to the requirement of sealers (Donnermeyer, Bürklein, et al., 2018). Bioceramic sealers used in endodontics are mainly composed of calcium-silicate. As with the terminology of other material which is based on the main component. Bioceramic sealed is also called “calcium-silicate based sealers” (CSBS). The CSBS consists of two groups sharing a common setting reaction. The first group is a premixed CSBS which require external water supply while the second group is a two-component CSBS which needs an internal water supply. List of the available CSBS in the market is shown in (Table 2.2) and (Table 2.3). CSBS setting reaction consists of two phases. The first reaction is a hydration reaction, which occurs in two different types (A+B) (Brave et al., 2012).



The hydration reaction is followed by a precipitation reaction of calcium phosphate:



Some other sealers found in the market contains bound calcium silicate. However, the biological effect of such material is doubtful (Camilleri et al., 2014).

Table 2.2 List of the available CSBS in the market (1-component)

Sealer	Manufacturer	Delivery	Composition
iRoot SP	Innovative Bioceramix, Vancouver, Canada	1-Component material	zirconium oxide, dicalcium silicate, tricalcium silicate, calcium phosphate monobasic, calcium hydroxide, filler
Endoseal MTA	Maruchi, Wonju, Korea		calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radio pacifier
Well-Root ST	Vericom, Gangwon-Do, Korea		calcium aluminosilicate, zirconium oxide, filler, thickening agent
Nano-Ceramic Sealer	B&L Biotech, Fairfax, USA		calcium silicates, zirconium oxide, filler, thickening agent
EndoSequence BC Sealer Hi-Flow	Brasseler USA, Savannah, USA		Until today no information about the composition, the manufacturer states it is a variation of Endosequence BC Sealer

Table 2.3 List of the available CSBS (2-components)

Sealer	Manufacturer	Delivery	Composition
BioRoot RCS	Septodont, Saint-Maur-des-Fossés, France	2-Components material	<i>Powder:</i> tricalcium silicate, zirconium oxide, povidone <i>Liquid:</i> aqueous solution of calcium chloride and polycarboxylate
Endo CPM	EGEO SRL, Buenos Aires, Argentina		<i>Powder:</i> mineral trioxide aggregate, bismuth oxide, barium sulfate, silica dioxide <i>Liquid:</i> aqueous solution of calcium chloride, sodium citrate, propylenglycolalginat, propylenglycol
Tech BioSealer Endo	Isasan SRL, Revello Porro, Italy		<i>Powder:</i> White Portland cement, bismuth oxide, anhydride, sodium fluoride <i>Liquid:</i> Alfacaine SP solution (4% arcaine + 1/100.000 epinephrine)
ProRoot ES	Dentsply, York, USA		<i>Powder:</i> tricalcium silicate, dicalcium silicate, calcium sulfate, bismuth oxide tricalcium aluminate <i>Liquid:</i> water, viscous water-soluble polymer

i **Properties of bioceramic sealers**

I. Physiochemical properties

a. Material characterization

To assess physical, chemical, mechanical and microstructural properties of materials, several tests can be performed. Energy-dispersive spectroscopy (EDS) is used to analyse the material elements, X-ray diffraction analysis (XRD) to characterize the crystalline phases in any given material and scanning electron microscopy (SEM) to examine the surface of the material. EDS results showed increased calcium and phosphate on the surface of iRoot SP, which was established to be a component of human hard tissue (Benezra et al., 2018). Furthermore, EDS analysis showed precipitation with a high concentration of calcium on the surface of iRoot SP when immersed in simulated body fluids (Carvalho et al., 2017). BioRoot RCS showed similar results (Prüllage et al., 2016). Environmental scanning electron microscopy (ESEM) showed a regular surface with dispersed granules of BC sealer when freshly mixed while EDS showed phosphorus calcium and zirconia. At twenty-eight days ESEM demonstrated a coating of spherules. EDS showed increased in calcium and phosphate while zirconia became undetectable (Zamparini et al., 2018).

b. Radiopacity

iRoot SP (Zamparini et al., 2018), BioRoot RCS (Prüllage et al., 2016), Endoseal MTA (Lee et al., 2017) and Endo CPM (Cañadas et al., 2014) fulfilled the ISO-norm 6876:2012 requirement of having radiopacity greater than 3 mm aluminium thickness.

c. Flow and film thickness

The ISO-norm 6876:2012 requirement of flow greater than 17 mm and film thickness less than 50 μm were fulfilled by iRoot SP (Zhou et al., 2013), Endo CPM (Tanomaru-

Filho et al., 2013), Endoseal MTA (Benezra et al., 2017). Khalil et.al reported that BioRoot RCS fell short of the ISO-norm for both flow and film thickness (Khalil et al., 2016).

d. Setting time

Contradictory results were published regarding the setting time of iRoot SP. The setting times ranged from 2.7 h (Zhou et al., 2013) up to 168 h (Loushine et al., 2011). One study reported that BC didn't set completely within 4 weeks (Lee et al., 2017). The setting time of both BioRoot RCS and Endoseal MTA was increased when immersed in Hank's balanced salt solution (HBSS) or Dulbecco's modified Eagle medium (DMEM) (Benezra et al., 2017).

e. Water sorption and solubility

ISO-norm 4049 recommends less than 40 mg/mm³ of water sorption after 28 days. iRoot SP (Zamparini et al., 2018), BioRoot RCS and Endoseal MTA (Benezra et al., 2017) did not exceed this limit. AH Plus showed less water sorption compared to bioceramic sealers (Benezra et al., 2017).

Studies showed varying results on water solubility of bioceramic sealers. While some results exceeded the ISO-norm of less than 3% weight loss after immersion in water for 24 h (Poggio et al., 2017) others demonstrated solubility within the 3% limit (Ersahan & Aydin, 2013; Urban et al., 2018). In comparison with epoxy-resin based sealers, bioceramic sealers showed higher water solubility (Benezra et al., 2017; Urban et al., 2018; Zhou et al., 2013).

f. pH value

Bioceramic sealers showed a pH above 12 upon setting (H. Zhang et al., 2009). iRoot SP demonstrated high pH over a period of 14 days in the immersion solution (Zamparini et al., 2018). This value of pH decreased by the end of a 28 days period (Tanomaru-Filho et al., 2017). BioRoot RCS (Poggio et al., 2017) and Endoseal MTA (Lee et al., 2017) showed similar results.

II. Biological properties

a. Biocompatibility

The traditional concept of biocompatibility is regarded as a lack of significant adverse reaction between the oral tissues (Browne, 1988). It is established now that some materials can induce a non-significant reaction with the host tissues which can aid in the healing of the oral tissues (Browne, 1994). Hence, it is an important characteristic to be in endodontic materials especially if they are used for pulp capping, perforation repair or as a retrograde filling material. Variable tests can be used to evaluate biocompatibility including both *ex-vivo* and *in-vivo*.

Several investigations reported good biocompatibility of iRoot SP (Alsubait et al., 2018; Zhang & Peng, 2015). It was found to be non-cytotoxic on periodontal ligament (PDL) fibroblasts (Rodríguez-Lozano et al., 2017). In addition to that, iRoot SP demonstrated lower cytotoxicity compared to AH Plus (Candeiro et al., 2016), to MTA Fillapex and to zinc-oxide eugenol-based sealers (Silva et al., 2016). iRoot SP demonstrated better biocompatibility upon contact with macrophages compared to MTA (Yuan et al., 2018). Studies on osteoblast-like cells not only showed no toxicity of iRoot SP on osteoblast-like cells but also demonstrated more production of mineralized matrix gene and protein expression in comparison with AH Plus (Zhang et al., 2010). Furthermore, in the presence of iRoot SP, human tooth germ stem cells manifested

differentiation into osteoblast-like cells (Güven et al., 2013). Similar results were reported with BioRoot RCS as it showed good biocompatibility with periodontal ligament cells (Jung et al., 2018) and gingival fibroblasts (Poggio et al., 2017). Furthermore, it showed less cytotoxicity in comparison to epoxy-resin based sealers on fibroblasts (Vouzara et al., 2018) and promoted the differentiation of human pulp stem cells (Loison-Robert et al., 2018). Endoseal MTA is more biocompatible compared to AH Plus (Lim et al., 2015) but it demonstrated inferior biocompatibility compared to BioRoot RCS and iRoot SP (Collado-González et al., 2017).

b. Antimicrobial activity

iRoot SP displayed anti-bacterial activity against *E. feacalis*, *Escherichia coli*, *Lactobacillus*, *Pseudomonas Aeruginosa*, *Staphylococcus* and *Candida Albicans* (G Singh et al., 2016). The antimicrobial activity of iRoot SP extends over a 7 days period (W. Zhang et al., 2009). A more pronounced anti-microbial activity was exhibited by iRoot SP and BioRoot RCS compared to AH Plus (Colombo et al., 2018; Gurpreet Singh et al., 2016). Furthermore, a study comparing the anti-microbial activity calcium hydroxide (CH), grey MTA, white MTA, Portland cement and CSBS cement manifested a higher growth inhibition zones around CH and CSBS. There was a significant difference between CSBS and MTA and Portland cement groups (Asgary & Kamrani, 2008).

c. Leakage

Contradictory results have been reported from the studies compared leakage using CSBS and AH Plus sealer. While some studies were in favour of iRoot sp (Ballullaya et al., 2017; Pawar et al., 2014), others were in favour AH Plus (Ulusoy et al., 2014). Furthermore, one study demonstrated similar results in term of the amount of leakage between the two sealers (W. Zhang et al., 2009). On the other hand, iRoot SP proved to

be superior in having better sealing ability compared with MTA (Gandhi & Halebathi-Gowdra, 2017).

d. Bioceramic sealer interaction with dentine

Endo sequence BC sealer demonstrated dentinal tubules penetration as deep as 2 mm (McMichael et al., 2016). Studies comparing Endo sequence BC to AH Plus indicate a significantly higher dentinal tubule penetration of Endo sequence BC sealer (Del Monaco et al., 2018; Wang et al., 2018). On the other hand, BioRoot RCS exhibited contradictory results on dentinal tubule penetration when it was compared with epoxy resin sealer AH Plus (Donnermeyer, Bürklein, et al., 2018).

ii Removal of bioceramic-based sealers

As the new generation of sealers has come to light, the ability to remove them from the root canal system is a matter of concern for clinicians. Therefore, several studies have been done to investigate the retreatability of bioceramic sealers using variable techniques and parameters.

The experiments investigated the removal of bioceramic sealers, the main filling material was gutta-percha. However, different studies used different obturation techniques. Root canals were obturated using single cone technique, lateral compaction and warm vertical compaction. Furthermore, two studies evaluated the retreatment efficacy when the master cone was deliberately 2 mm short of the working length (Agrafioti et al., 2015; Hess et al., 2011). The ability to reach the working and patency was also investigated by multiple studies. The results ranged from 14% (Oltra et al., 2017) to 100% (Donnermeyer et al., 2018)

A wide variety of techniques have been used in the assessment of bioceramic sealer removal. Rotary and hand instrument were used in conjugation with sodium hypochlorite

and Ethylenediaminetetraacetic acid (EDTA) in order to remove the root filling material (Hess et al., 2011; Suk et al., 2017). While studies compared the efficacy of removing bioceramic root filling using hand and rotary instruments (Ersev et al., 2012) others compared the efficacy of different rotary systems (Donnermeyer et al., 2018). No significant difference was found.

Contradictory results have been reported regarding the dissolving capacity of endodontic solvents on bioceramic sealers. While Oltra et al. (2017) found the use of chloroform beneficial and significantly reduced the residual bioceramic sealer, Ma et al. (2012) reported that chloroform delayed the removal of iRoot SP from the root canal.

Remnant material after removal of bioceramic sealer was evaluated using various techniques such as digital radiography (Ersev et al., 2012), scanning electron microscopy (Hess et al., 2011), confocal microscopy (Kim et al., 2015), micro CT (Suk et al., 2017) and optical microscopy (Donnermeyer, Bunne, et al., 2018) .

The remnants of bioceramic sealers have been compared with those of AH Plus, AH 26, MTA Fillapex, Hybrid Root SEAL, Active GP system, MM Seal, Pulp Canal Sealer EWT and Endo CPM after removal procedures. Upon comparison of Endosequence BC sealer with AH Plus studies reported similar remnants associated with both sealers (Ersev et al., 2012; Kim et al., 2015; Simsek et al., 2014; Suk et al., 2017) or less (Oltra et al., 2017) while one study reported more remnants associated with Endosequence BC sealer (Uzunoglu et al., 2015). Upon comparing the residual material after retreatment between Endosequence BC sealer, Hybrid Root SEAL and Active GP no significant difference was reported (Ersev et al., 2012). Similar results were also associated with iRoot SP - MM Seal (Simsek et al., 2014) and BioRoot RCS – Endo CPM (Donnermeyer, Bunne, et al., 2018). MTA Fillapex was reported to have significantly less debris than iRoot Sp (Suk et al., 2017). However, Donnermeyer, Bunne, et al. (2018). and Uzunoglu et al.

(2015) disagreed that this sealer leaves the same amount as iRoot SP (Donnermeyer, Bunne, et al., 2018; Uzunoglu et al., 2015). BioRoot RCS was reported to have fewer remnants than AH plus (Donnermeyer, Bunne, et al., 2018) The inconsistency of the results might be imputed on the different methodologies such as the use of different types of teeth and the methods of evaluating the remaining material. Available studies on removal of CSBS are listed in (Table 2.4)

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Table 2.4 Studies referring to the removal of CSBS

Study	Sample	Sealer	Evaluation method	Results
Hess et al. (2011)	40 mesiobuccal roots of lower molars	AH Plus Endosequence BC sealer	WL and P T	The group obturated with BC sealer 2 mm short of WL only 30% of the sample could achieve WL and P. All the -sample of other groups achieved WL and P.
Ersev et al. (2012)	120 palatal roots of upper molars	Active GP system AH Plus, Endosequence BC Hybrid Root SEAL	Digital X-ray	Irrespective of the sealer type and retreatment technique, filling material could not be removed completely from the root canals. The most residues were in the apical third
Ma et al. (2012)	40 lower incisors	iRoot SP	Micro-CT Time	In the nonsolvent groups, less time was needed to achieve satisfactory gutta-percha removal and root canal refinement than in the solvent groups.
Neelakantan et al. (2013)	45 single-rooted teeth	MTA Fillapex MTA Plus AH Plus	CBCT Time	The least remaining root filling material was demonstrated by MTA Fillapex
Simsek et al. (2014)	60 single-rooted premolars	AH Plus iRoot SP MM Seal	Time SEM	There was no difference between the sealers and between retreatment techniques
Carpenter et al. (2014)	86 maxillary anterior teeth	MTA Fillapex	P	Chloroform, Endosolv E, and Eucalyptol soften GP and MTA Fillapex sufficiently to aid in re-establishing apical patency during endodontic retreatment
Uzunoglu et al. (2015)	40 lower premolars	AH 26 iRoot SP MTA Fillapex	SM Time	single-cone GP/iRoot SP presented significantly more remaining filling material than single-cone GP/AH-26
Kim et al. (2015)	28 single-rooted teeth	AH Plus EndoSequence BC Sealer	SEM, CM P,T	The present study shows that Endo-Sequence BC sealer and AH Plus sealer have similar efficacy in dentin penetration and retreatment

Study	Sample	Sealer	Evaluation method	Results
Agrafioti et al. (2015)	54 single-rooted teeth	AH Plus MTA Fillapex TotalFill BC Sealer	OM WL and P T	The WL and patency were reestablished in 100% of specimens in all groups.
Zuolo et al. (2016)	64 lower canines	Endosequence BC Pulp Canal Sealer EWT	Micro-CT P T	Endosequence BC sealer groups exhibited significantly more remaining filling material in the canals and required more time for retreatment.
Oltra et al. (2017)	56 upper incisors	AH Plus Endosequence BC Sealer	Micro-CT P	BC Sealer had significantly more residual filling material than the AH Plus. In the group retreated without chloroform, patency could only be re-established in 14%. Significantly less than the other groups.
Suk et al. (2017)	36 upper lateral incisors and lower premolars	AH Plus Endosequence BC Sealer MTA Fillapex	Micro-CT P	No differences in the amount of the remaining filling material between EndoSequence BC and the AH Plus groups. The photon-initiated photoacoustic streaming improved the removal of filling remnants in all groups
Donnermeyer, Bunne, et al. (2018)	192 single-rooted teeth	AH Plus BioRoot Endo C.P.M MTA Fillapex	LM T	Fewer sealer remnants and shorter retreatment times were observed in CSBS Retreatment with engine-driven NiTi instruments was superior compared to hand instrumentation.
Kontogiannis et al. (2019)	120 single rooted lower premolars	MTA Fillapex, TotalFill BC Sealer, AH Plus	P	WL was fully regained in all cases. Patency regaining was easier in AH+ Remnants were significantly more in TotalFill groups.

2.5 Root canal retreatment

2.5.1 Aetiology of root canal failure

Failure of root canal treatment is defined as the persistence of peri-apical radiolucency following root canal treatment (Nair, 2004b). Most of the failure cases are a consequence of a root canal treatment falling below the acceptable standards (Sjögren, 1996). Commonly associated problems with endodontic failure include inadequate disinfection control, poor access cavity design, unexploited canals, improper instrumentation, and inadequate temporary and permanent fillings (Sundqvist et al., 1998). Clinicians can be deceived by the belief that procedural errors such as separated instruments, ledges, perforations and many more are the direct cause of endodontic failure (Lin et al., 2005). However, procedural errors do not imperil the outcome of the endodontic treatment unless it is associated with an infection. In fact, a procedural error can make proper intracanal procedure unachievable. Thus, there is a chance for the failure of the root canal upon dealing with a procedural error during root canal treatment (Siqueira Jr, 2001).

Causes of endodontic failure can be classified by the source of the irritant as intraradicular infection, extra-radicular infection, cystic apical periodontitis and foreign body reaction (Nair, 2004b). It is established that the root canal system cannot be completely cleaned during chemo-mechanical preparation with the available instruments and techniques (Lin et al., 1991; Siqueira et al., 1997). The underprepared area of the root canal system may contain not only remnants of necrotic tissue but also it may contain bacteria even if the root canal filling seems to be adequate radiographically (Nair, Sjögren, Krey, Kahnberg, et al., 1990). Bacteria located in areas such as ramification, deltas and irregularities will not only have a chance of surviving the endodontic aseptic procedure but also their nutrient supply most probably will remain unaltered (Siqueira Jr, 2001). On the opposite side, bacteria entombed by the root canal filling usually die or prevented from getting access to the peri-radicular tissue. However, even if entombed

some bacteria species will survive for a long time. So, if the root canal filling fails to properly seal the root canal space, leakage of tissue fluids might provide nutrient for bacterial growth. If the growing bacteria reach the threshold number it might cause a periapical infection (Siqueira Jr, 2001).

Extra-radicular infection can happen in cases of acute apical periodontitis lesion (Ramachandran Nair, 1987), periapical actinomycosis (Sjögren et al., 1988; Sundqvist & Reuterving, 1980), extrusion of infected root fragments into the periapex (Yusuf, 1982) and infected periapical cysts with cavities open to the root canal (Nair, 1987). Foreign body reaction can be induced by endogenous cholesterol crystal deposited in the periapical tissue. Studies reported accumulation of macrophages and giant cells around cholesterol crystals which suggests a typical foreign body reaction (Sjögren et al., 1995). Macrophages and giant cells surrounding the cholesterol crystal are not only unable to degrade the cholesterol crystal but also a source of inflammatory and bone-resorptive mediators (Sjögren et al., 2002). Therefore, the presence of cholesterol crystal in the periapical area can prevent the healing of periapical tissue after conventional root canal treatment (Nair, 2004b). Exogenous material can also induce foreign body reaction if trapped in the periapical tissue (Koppang et al., 1992). Gutta-percha is the most widely used core material for orthograde root canal treatment. The concept of gutta-percha as a well-tolerated biocompatible material is debatable as studies reported delayed healing in the case of extruded gutta-percha into the periapical tissue (Kerekes & Tronstad, 1979; Ng et al., 2011; Sjögren et al., 1990). It has been demonstrated that large pieces of gutta-percha are well encapsulated while minuscule particles of gutta-percha induce an intense, localized reaction associated with macrophages and giant cells (Sjögren et al., 1995). Furthermore, contaminated gutta-percha cones can induce a foreign body reaction at the periapical tissue (Nair, Sjögren, Krey, & Sundqvist, 1990). Occasionally, periapical tissue

that heals with scar tissue can be mistaken as a radiographic sign of root canal treatment (Nair et al., 1999).

2.5.2 Prevalence of root canal treatment failure

A number of studies investigated the success of primary root canal treatment and factors that affect the outcome. Ng et al. (2007) conducted a systematic review of the available studies investigated the outcome of primary root canal treatment. A total of 63 studies were included from 1992-2002. They reported a failure rate ranging from 15% - 25% depending on the criteria of success whether strict or loose (Ng et al., 2007). However, most of the studies included were cohort or retrospective studies, therefore the level of evidence was low (Ng et al., 2007). To increase the level of evidence few prospective studies have been conducted. de Chevigny et al. (2008) performed a series of studies (Toronto study) and reported an overall failure rate of 15% (de Chevigny et al., 2008). Another prospective study was conducted and reported a failure rate of 17% (Ng et al., 2011).

It is important to mention that most of the studies are standardized and done by experienced or supervised by experienced endodontists which might not reflect the normal clinical situation. Therefore, the conduction of epidemiological studies was necessary. Epidemiological studies performed in different populations reported a high prevalence of apical periodontitis in connection with root-filled teeth ranging from 27% to 72% (Al-Omari et al., 2011; Huumonen et al., 2017; van der Veken et al., 2017).

2.5.3 Treatment after the failure of primary root canal treatment

Treatment options after failure of primary root canal treatment include nonsurgical treatment, endodontic surgery, tooth replantation, transplantation, extraction and replacement by an implant or fixed prosthesis and extraction without replacement (Torabinejad & White, 2016). From the point view of health care economics, an

alternative to retaining the natural dentition must result in lower lifetime costs or provide greater lifetime function, freedom of disease and acceptability of the patient (Torabinejad et al., 2007). On the hand, for a natural tooth to be retained it must not have the residual disease of clinical significance, must satisfy its functional and aesthetic requirement and be comfortable (Torabinejad & White, 2016). Studies reported a high success rate of nonsurgical root canal retreatment as reported by the Toronto study in which cases treated with nonsurgical endodontic treatment had 93% survival rate with function (Farzaneh et al., 2004). Another study reported a 95% per cent 4-year survival rate (Ng et al., 2011). Hence, nonsurgical root canal retreatment is considered the first treatment option after failure of primary root canal treatment (Torabinejad & White, 2016). Furthermore, nonsurgical root canal retreatment reported a more favourable outcome in the long run compared to endodontic surgery (Torabinejad et al., 2009). In some cases, the nonsurgical retreatment for failed teeth might not be feasible and alternative treatment options should be considered.

Recently, the outcome of endodontic surgery improved drastically because of the introduction of the microscope, angled ultrasonic instruments and new retrograde grade filling materials. Studies reported 4 to 6 years of survival rates of 88% of teeth treated with microsurgical techniques (Torabinejad et al., 2015). Tooth replantation is the insertion of a tooth into its own alveolus after the tooth has been extracted for the purpose of performing treatment, such as root filling or perforation repair (AAE, 2015). Transplantation is defined as the transfer of embedded, impacted or erupted tooth from one site to another socket or surgically prepared socket either in the same or another person (AAE, 2015). Ankylosis and resorption are common with replanted and transplanted teeth. Hence, such treatment is indicated when there is no other modality is available to maintain a strategic tooth (Torabinejad & White, 2016).

2.5.4 Assessment methods for techniques used to remove canal filling material

The following methods have been used to assess the efficacy of the different techniques used to remove root filling material:

- 1- Tooth sectioning
- 2- Tooth clearing
- 3- Radiographic assessment

2.5.4.1 Tooth-sectioning

Tooth sectioning is a process in which teeth are sectioned vertically into halves to visualize the root canal. Subsequently, the separated halves are evaluated to qualitatively measure the remaining debris using different methods such as photographing the sectioned halves directly or under a microscope. Then these records can be evaluated by one or more operator (Zmener et al., 2006). Other studies combined tooth-sectioning technique with scanning electron microscopy (Somma et al., 2008) and image analysis software (Hassanloo et al., 2007) for better and more accurate results.

2.5.4.2 Tooth clearing

Tooth clearing gives a three-dimensional view of the root canal space in relation to the exterior of the teeth and allows its examination. This method has been used to assess the morphology of the root canal system (Omer et al., 2004), the quality of root canal filling (Venturi & Breschi, 2004) and the efficacy of root canal filling material removal (Schirrmeister et al., 2006). This method uses a strong acid such as hydrochloric acid (Omer et al., 2004) or nitric acid (Schirrmeister et al., 2006) to decalcify the tooth followed by ascending concentration of alcohol to dehydrate tooth. In the end, the teeth get immersed in methyl salicylate to make transparent. The clear teeth then get photographed with a digital camera or stereomicroscope and then the residue is compared using image analysis software (Taşdemir et al., 2008).

2.5.4.3 Radiographic assessment

Radiographic imaging for studying the outcome of canal instrumentation is a non-destructive and reproducible technique which makes it a valuable tool in endodontic research (Southard et al., 1987). Nowadays there are two main categories of radiographic imaging.

(a) *Two-dimensional imaging*

Periapical imaging is the most common type of radiographic assessment used in endodontics. It uses conventional periapical film or digital sensor with standardized exposure time and distance between the x-ray source and the x-ray film. Then the processed radiographs can be assessed on a viewer or get digitalized with a scanner to be assessed by software to measure the residual material inside the root canal system (Masiero & Barletta, 2005). However, these images provide limited information due to several reasons:

***i* Compression of three-dimensional anatomy**

Periapical radiographs compress 3-dimensional anatomy into 2-dimensional image limiting its diagnostic ability (Cohenca et al., 2007). Therefore, the position, nature and geometry of structure within the root canal system are difficult to assess. (Cohenca et al., 2007; Patel & Dawood, 2007). In order to avoid this problem, it was suggested to take more than one view with a different head angle (parallax technique) (Glickman & Pettiette, 2006; Patel & Ford, 2007). However, even with multiple exposures, complete identification of relevant structures within the root canal system is not guaranteed (Matherne et al., 2008).

ii **Geometric distortion**

Over angulated or under angulated radiographs may affect the root length in the developed image (White & Pharoah, 2004). Even in ideally taken periapical radiographs, it is anticipated to have some degree of distortion (Forsberg & Halse, 1994).

Another disadvantage of periapical radiographs is the presence of fine layers of debris not radiopaque enough to be detected (Ferreira et al., 2001) which gives an impression of false cleanliness (Betti & Bramante, 2001).

(b) **Three-dimensional imaging**

Since the introduction of X-ray computed tomography (CT) imaging in the early 1970s, the practice of medicine was revolutionized. It provided advanced diagnostic imaging collected from multiple viewing angles producing three-dimensional (3D) spatial construction showing maps of material density within attenuating material or tissues such as teeth (Hounsfield, 1973).

i **Cone-beam computed tomography (CBCT)**

It was developed in the late 1990s to capture 3-dimensional images of the maxillo-facial skeleton at a lower radiation dose compared to CT scans (Arai et al., 1999). CBCT scanners are straightforward to use and more compact compared to computed tomography (CT) scanners which make it suitable for dental use (Scarfe et al., 2006). Tomographic slices produced as thin as one voxel can be viewed in coronal, axial and sagittal planes simultaneously allowing the clinician to have a truly three-dimensional view of the tooth. The limitations of using CBCT are related to the cone beam geometry, the sensitivity of the sensor and the contrast resolution that produce images of less quality and utility conventional CT images. Furthermore, three types of artefacts can be produced while using CBCT. The first type is partial volume averaging which happens when the selected voxel size is larger than the object being scanned. The second type is undersampling

which happens when the number of projections used for image reconstruction is low which leads to noisier images. The last type is the cone-beam effect which happens mainly at the peripheral portions due to the divergence of the x-ray beam during rotation around the patient. This type of artefact results in image distortion, streaking and greater peripheral noise.

ii **Micro-computed tomography**

Typically, clinical CT scanner is capable of producing images composed of 1 mm^3 volume element (voxels) while X-ray microcomputed tomography (Micro-CT) introduced in the early 1980s produced a much better spatial resolution. It produces voxels in the range of 5-50 μm , approximately 1,000,000 times smaller in volume than CT voxels (Kuhn et al., 1990). Micro-CT systems use a micro focal spot x-ray source and high-resolution detector, allow for projections rotated through multiple viewing directions to produce 3D reconstruction images of samples. A wide variety of specimens including both mineralized and soft tissues can be studied using Micro-CT.

Micro-CT imaging is a non-destructive procedure allows for repeated exposure and acquisition of information (Crozeta et al., 2016). Furthermore, it provides accurate 3-dimensional models and acquisition of quantitative data (Versiani et al., 2016). Therefore, the studied sample can be scanned many times and still be viable for additional biological and mechanical testing (Swain & Xue, 2009). For this reason, micro-CT studies are widely used in many academic fields and dentistry is no exception. Micro-CT is an invaluable research tool in endodontics *in-vitro* studies. It has been used to analyse canal morphology (Peters et al., 2000), canal preparations (Cheung & Cheung, 2008), quality of root canal treatment (Iglesias et al., 2017) and to evaluate the removal of root filling material (Oltra et al., 2017). On the other hand. Micro-CT carries a few limitations and

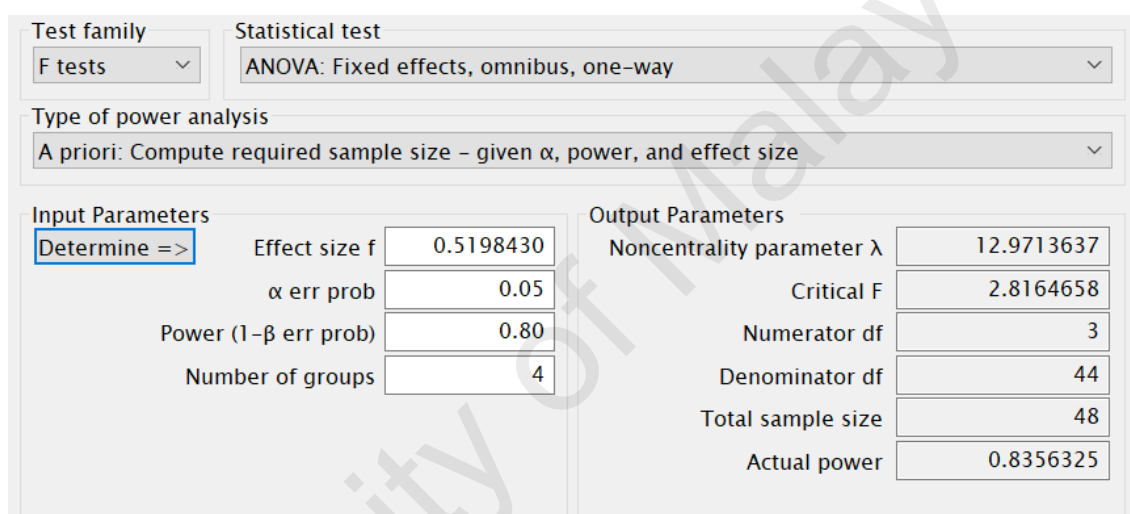
drawbacks. It is expensive, delivers a high dose of radiation, it can be misinterpreted and cannot correlate radiographic and histologic findings (Fred, 2004).

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CHAPTER 3: MATERIALS AND METHODS

3.1 Sample calculation:

The sample size was calculated using G-power software with the following parameters, α -error probability 0.05, power (1- β) 0.80, standard deviation (SD) = (3.6) and the following means (5.70,1.71,2.90,0.70). Input Parameters from Hammad et al. (2008b) study was used to estimate the sample size. The sample size output was 48, 12 in each group (Figure 3.1). Extra 25 % of the sample was added as a compensation for potential sample loss. Hence, the total sample is 60.



The screenshot shows the G-power software interface for sample size calculation. The 'Test family' is set to 'F tests' and the 'Statistical test' is 'ANOVA: Fixed effects, omnibus, one-way'. The 'Type of power analysis' is 'A priori: Compute required sample size - given α , power, and effect size'. The 'Input Parameters' section includes: 'Determine =>' (selected), 'Effect size f' (0.5198430), ' α err prob' (0.05), 'Power (1- β err prob)' (0.80), and 'Number of groups' (4). The 'Output Parameters' section includes: 'Noncentrality parameter λ ' (12.9713637), 'Critical F' (2.8164658), 'Numerator df' (3), 'Denominator df' (44), 'Total sample size' (48), and 'Actual power' (0.8356325).

Input Parameters		Output Parameters	
Determine =>	Effect size f	Noncentrality parameter λ	12.9713637
	α err prob	Critical F	2.8164658
	Power (1- β err prob)	Numerator df	3
	Number of groups	Denominator df	44
		Total sample size	48
		Actual power	0.8356325

Figure 3.1 G-power sample size calculation

3.2 Teeth collection and storing:

Hundred three extracted single-rooted mandibular premolar teeth were collected with no age or race restrictions. The teeth were disinfected using 0.5% chloramine T trihydrate solution for one week. The soft and hard tissue remnants were removed using an ultrasonic scaler (Peizon[®] Master 400, Switzerland). Afterwards, the teeth were stored in 4°C distilled water.

3.3 Teeth selection

The collected teeth were examined under stereomicroscope (Kyowa Optical, Japan) at 10x magnification. Teeth were inspected for cracks, caries, external resorption and fractures. Then, teeth were scanned using cone beam computed tomography (CBCT)

machine Kodak 9000 (Carestream Kodak Co., New York, USA). The selection criteria were as the following: teeth with single canal, long oval canal, completely formed apex with patent foramina, canal curvature of less than 20° , no internal resorption or obstacle within the root canal system. The teeth which didn't comply with the inclusion criteria were excluded.

3.3.1 Canal curvature calculation

Using the CBCT scans the canal curvature was measured as per Schneider's technique (Schneider, 1971), a line was drawn on the radiograph parallel to the long axis of the canal. A second line was drawn from the apical foramen to intersect with the first line at the point where the canal started to leave the long axis of the tooth (Figure 3.2). The acute angle formed was then measured using CS 3D Imaging software. An example is shown in (Figure 3.3)



Figure 3.2 Schneider's technique canal angle determination

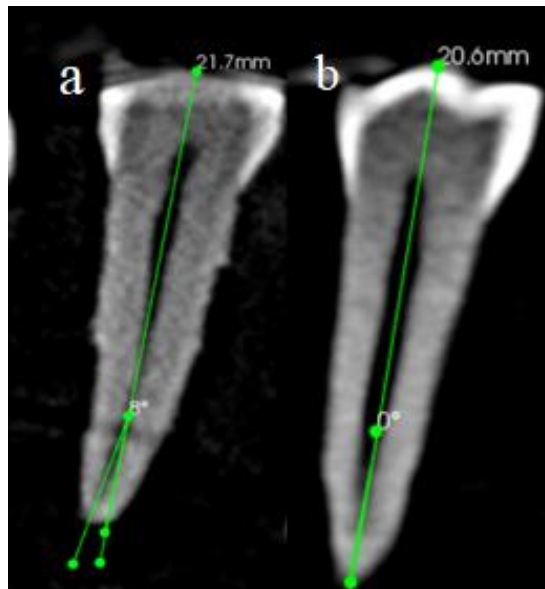


Figure 3.3 Canal angle calculation.a- mesiodistal view,b- buccolingual view

3.3.2 Canal shape determination

Using CBCT scans the shape of canals was recorded 6 mm coronal to the apex using CS 3D Imaging software (Paqué & Peters, 2011). The relation between the maximum initial horizontal dimension (MaxIWW) and minimum initial horizontal dimensions (MinIWW) was used to determine the shape of the canal according to Jou et al. (2004) classification (Jou et al., 2004) (Figure 3.4). An example is shown in (Figure 3.5).

Round canal: MaxIWW equals MinIWW.

Oval canal: MaxIWW is greater than MinIWW (up to two times)

Long oval canal: MaxIWW is 2 or more times greater than MinIWW (up to 4 times).

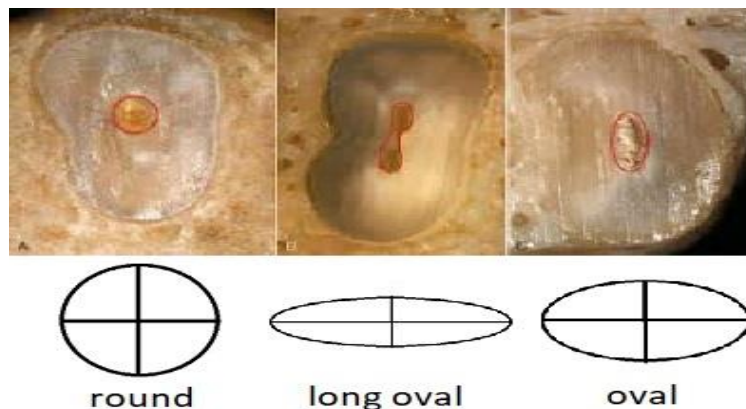


Figure 3.4 Canal shape classification

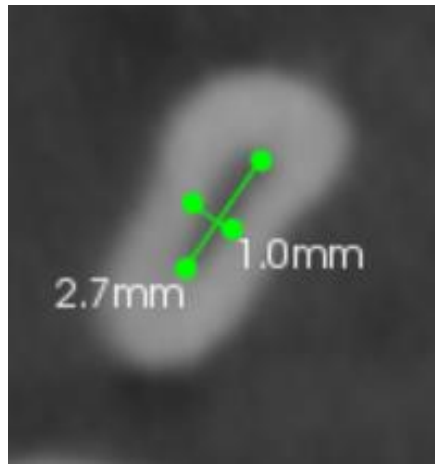


Figure 3.5 Canal shape determination, 6 mm from the apex

3.4 Teeth preparation and Instrumentation of the root canal system

A total of 48 teeth were selected. The teeth were decoronated with diamond disk (Bego, Germany) at length of 13 mm to standardize the samples (Figure 3.6). Working length (WL) was established 1 mm short of the root length (12 mm). K-files (Dentsply Maillefer, Switzerland) size (10, 15) were used to achieve WL. The canals were prepared using ProTaper Next[®] system (Dentsply Maillefer, Switzerland) to size X3 (30/0,06). All rotary files were operated using an engine-driven motor VDW.SILVER[®] RECIPROC[®](VDW, München, Germany) according to the manufacturer instruction (Figure 3.7). K-file size 10 was used to reconfirm patency throughout the procedure. After each instrument Irrigation with 5.25% sodium hypochlorite (NaOCl) was performed and patency reconfirmed. A 30-G side-vented needle was used with all irrigation solutions to the working length. The final irrigation regime for each canal was 3 ml 5.25% NaOCl followed by 3 ml of 17% ethylenediaminetetraacetic acid (EDTA) (SmearClear[™], SybronEndo, Orange, USA) and 3 ml 5.25% NaOCl to remove the smear layer. The cleaning and shaping procedure was terminated when the canal walls felt smooth and the master apical file (#30) could reach the working length without any obstruction. All the canals were dried with paper points and patency reconfirmed before obturation.

All canals were obturated using the hydraulic technique. The iRoot[®] SP (Innovative Bioceramics, Vancouver, Canada) sealer comes in premixed tubes and was introduced into the canals using lentulo spiral until the sealer was extruded through the apical foramen. ProTaper Next[®] gutta-percha cone Size 30 (0.06) was trimmed apically to fit 2 mm short from the working length, coated with the sealer, and then introduced into the canal to mimic a short root canal filling which will allow re-establishing patency to be achieved through the sealer. The access cavities were covered with Fuji II LC (GC America, Alsip, IL) then the teeth were stored in Memmert incubator IN450 (Mettler, Germany) at 37°C and 100% relative humidity for two weeks (Figure 3.8). 48 samples were elected out of the 60 based on the quality of the obturation. The obturated was assessed using buccolingual and mesiodistal periapical view (Figure 3.9).

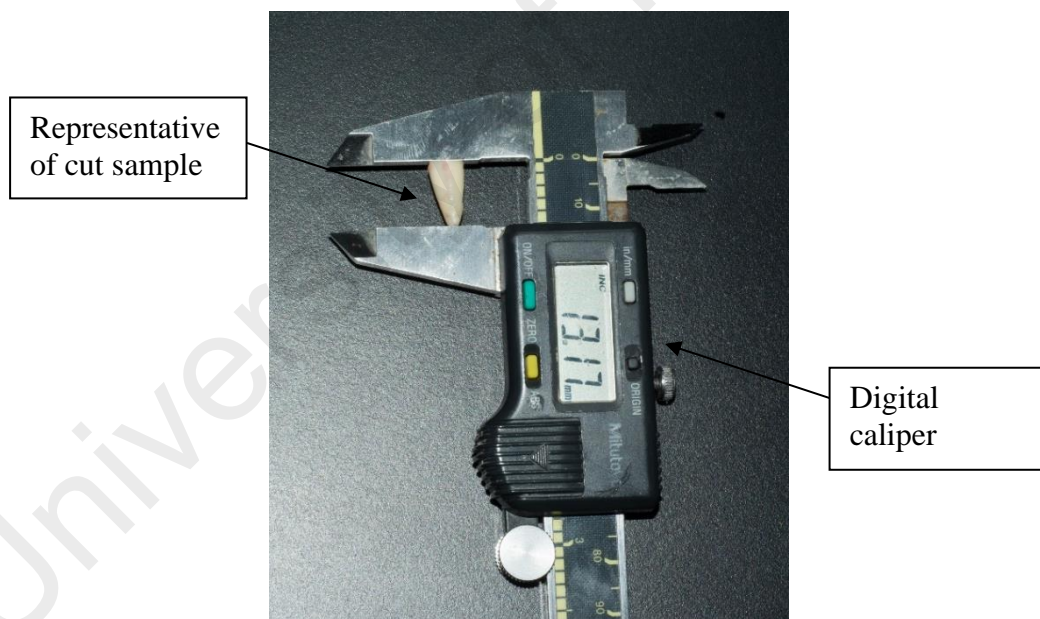


Figure 3.6 A lower premolar cut to the length of 13 mm to standardize the sample. the length checked with digital calliper



Figure 3.7 VDW Silver Reciproc



Figure 3.8 Memmert incubator IN450, The sample was stored at 37° with 100% relative humidity

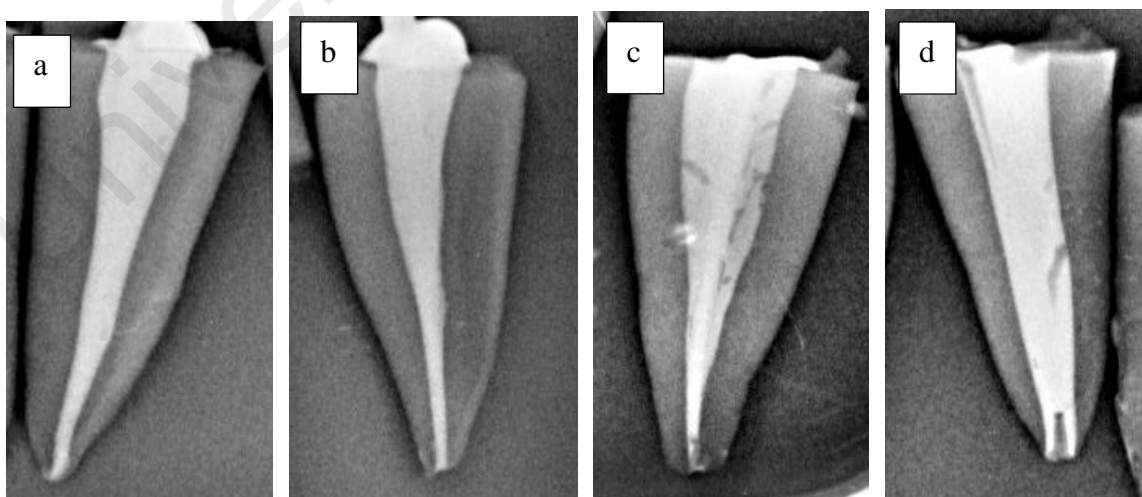


Figure 3.9 Example of included and excluded teeth after obturation. a and b show mesiodistal and buccal lingual periapical views of included sample. c and d show mesiodistal and buccolingual periapical views of excluded sample.

3.5 Initial micro-CT scanning

The samples were scanned after the completion of the obturation process using Xradia 520 Versa (Zeiss, Germany) micro-CT machine at the Department of Geology, University of Malaya, Kuala Lumpur, Federal Territory of Kuala Lumpur, Malaysia. The μ CT parameters were (18 μ m) voxel size, 70 kVp, 357 mA, 0.5 mm aluminium filter, angular rotation step 0.49°, 360° scanning (Table 3.1). The samples were fixed in a cylindrical shape of beading wax, fitted and mounted into the holder before scanning to reduce any possible movements during the scan. A sample of the scan is shown in (Figure 3.10). All the data was analysed using Drishti software Version 2.6.4.

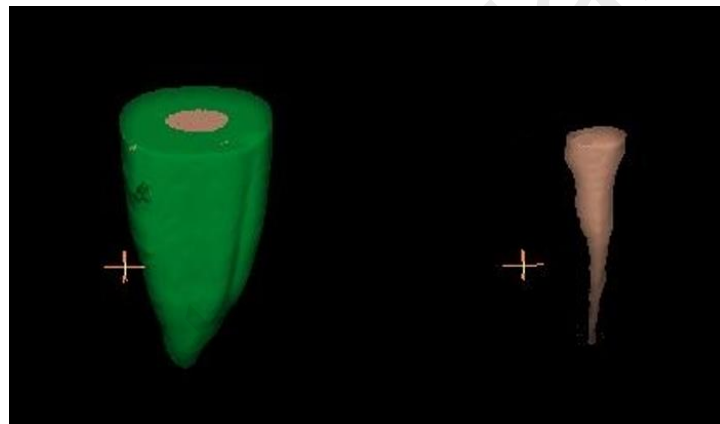


Figure 3.10 3-Dimensional reconstruction of one of the samples after the obturation

Table 3.1 Parameters used to scan the sample

ZEISS Xradia 520 versa parameter used to scan the sample	
Source voltage(kv)	80
Source current(uA)	88
Image pixel size (μ m)	18.4
No. of slices	1601
Rotational angle	360 Degree
Rotational angle	0.82 Degree
Exposure time (seconds)	1.5

3.6 Sample randomization

The sample was randomized into 4 groups with 12 samples in each group using research randomizer software

Table 3.2). After randomization, the homogeneity of the samples within each group was tested ($P= 0.115$). The volume difference between the groups was also tested ($P=0.755$). For both tests, P was significant at ($P<0.05$).

Table 3.2 Groups retreatment techniques

Group	n	Removal technique
1	12	Rotary files only
2	12	Rotary files and Xylol (5 min)
3	12	Rotary files and ultrasonic instrument
4	12	Rotary files, Xylol and ultrasonic instrument

3.7 Retreatment procedure

The coronal 2 mm of the gutta-percha was removed from all the samples using BeeFill (VDW, München, Germany) system to facilitate the introduction of the instruments and to act as a reservoir for Xylol. Each sample from all groups was negotiated using K-files size (10,15). Protaper[®] universal retreatment files (Dentsply Maillefer, Switzerland) was used for groups 1 and 2 according to manufacturer instructions until WL is reached or resistance is met. Additionally, for group 2, Xylol was applied to the canal for 5 minutes prior to using the Protaper[®] retreatment files. For all the samples, the removal procedure was terminated when there are no remnants of the filling material on the file's flutes (Figure 3.11). For all the groups, after the removal of root filling material with the

designated technique the canals were negotiated using K-file size 10 to achieve patency. The canal was considered patent if the file extends 1 mm beyond the WL. Afterwards, If the WL was reached, the canals were prepared using Protaper Next[®] (Dentsply Maillefer, Switzerland) (40,.06) to remove remaining obturation material. If the WL was not achieved, K-files size (10, 15) it was prepared to the reached length.

For group 3 and 4, the Protaper[®] universal retreatment files were also used according to manufacturer instructions until 2 mm short of the WL. The ultrasonic instrument, Endo Success[™] Retreatment Kit, ET25 tip (Acteon, England) was used according to manufacturer instruction using Newtron[®] P5 XS (Satelec Acteon, France) piezoelectric ultrasonic machine at speed of 10 on the walls and K-files (10,15) was used to reach full WL. ET 25 tip was also used on the walls with copious irrigation in order to pulverize the root filling material and remove the excess. When WL is achieved, the canal was prepared with Protaper Next[®] (Dentsply Maillefer, Switzerland) file (40,0.06). Similarly, if the WL is not achieved, the canal was prepared to the reached length.



Figure 3.11 Example of the root canal filling remnants on D3 Protaper universal retreatment file flutes

3.8 Final micro-CT scan

To evaluate the efficacy of the retreatment, all samples were scanned after obturation and after the completion of the retreatment process using a micro-CT machine Xradia 520 Versa (Zeiss, Germany) at the Department of Geology, University of Malaya, Kuala Lumpur, Federal Territory of Kuala Lumpur, Malaysia with the same parameters used in the initial scanning (Table 3.1). Complete scanning of one of the samples is shown in (Figure 3.12).

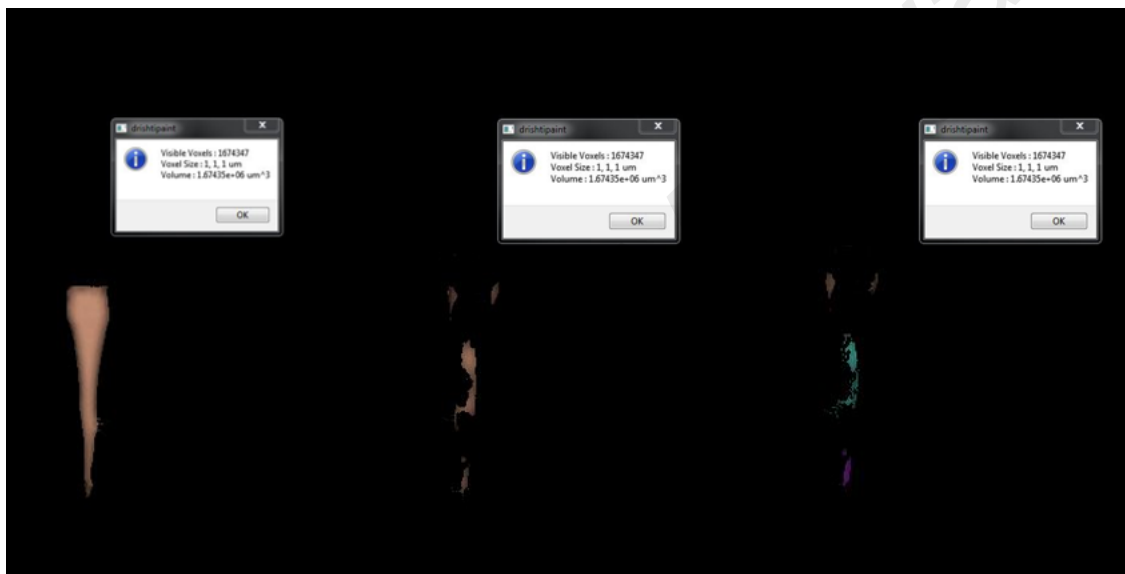


Figure 3.12 3-D reconstruction and volumetric measurements of one of the samples before and after removal of the root filling material.

3.9 Data analysis

The data was analysed using SPSS version 25 (IBM, USA), The normality of data was tested using Shapiro-Wilk test. As most of the groups were normal ($P > 0.05$) parametric statistical tests were conducted.

Objective	Test
To determine the percentage of remaining root filling material in long oval canals using 4 different techniques	One-way ANOVA
To determine the amount of remaining filling material in the coronal, middle and apical thirds of the canals between groups using micro-CT	One-way ANOVA
To determine the amount of remaining filling material in the coronal, middle and apical thirds of the canals within groups using micro-CT	Repeated measures ANOVA
To assess the ability to re-establish patency in canals obturated with iRoot SP(bioceramic sealer) and GP using RF, RF+Xylol, RF+UI, RF+Xylol+UI	Chi-square test

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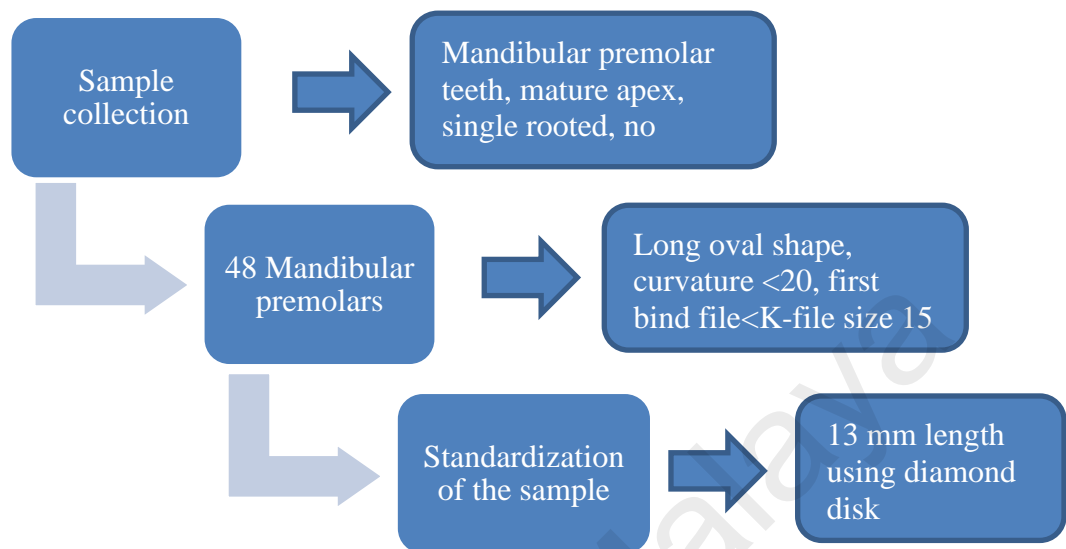


Figure 3.13 Sample selection and standardization

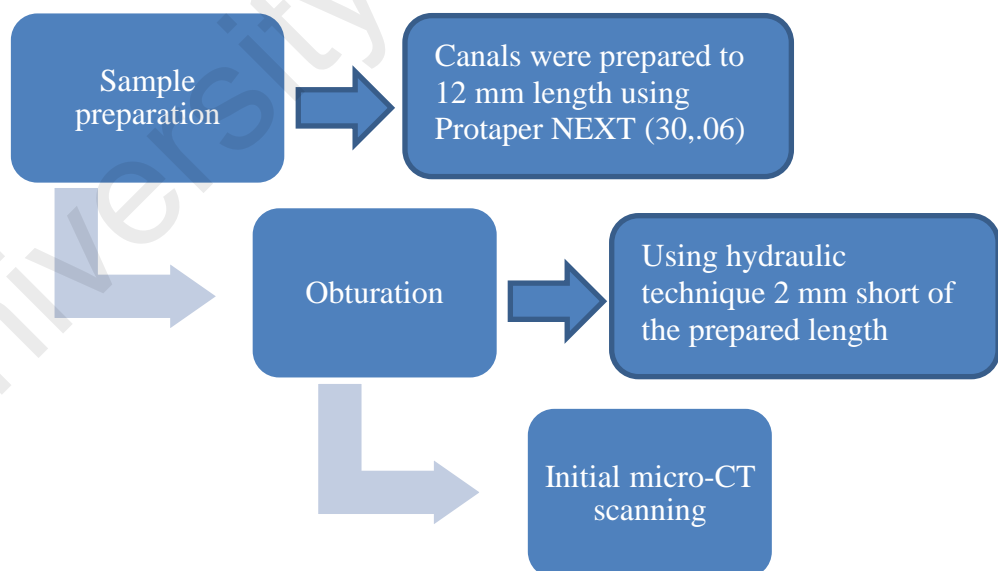


Figure 3.14 Sample preparation

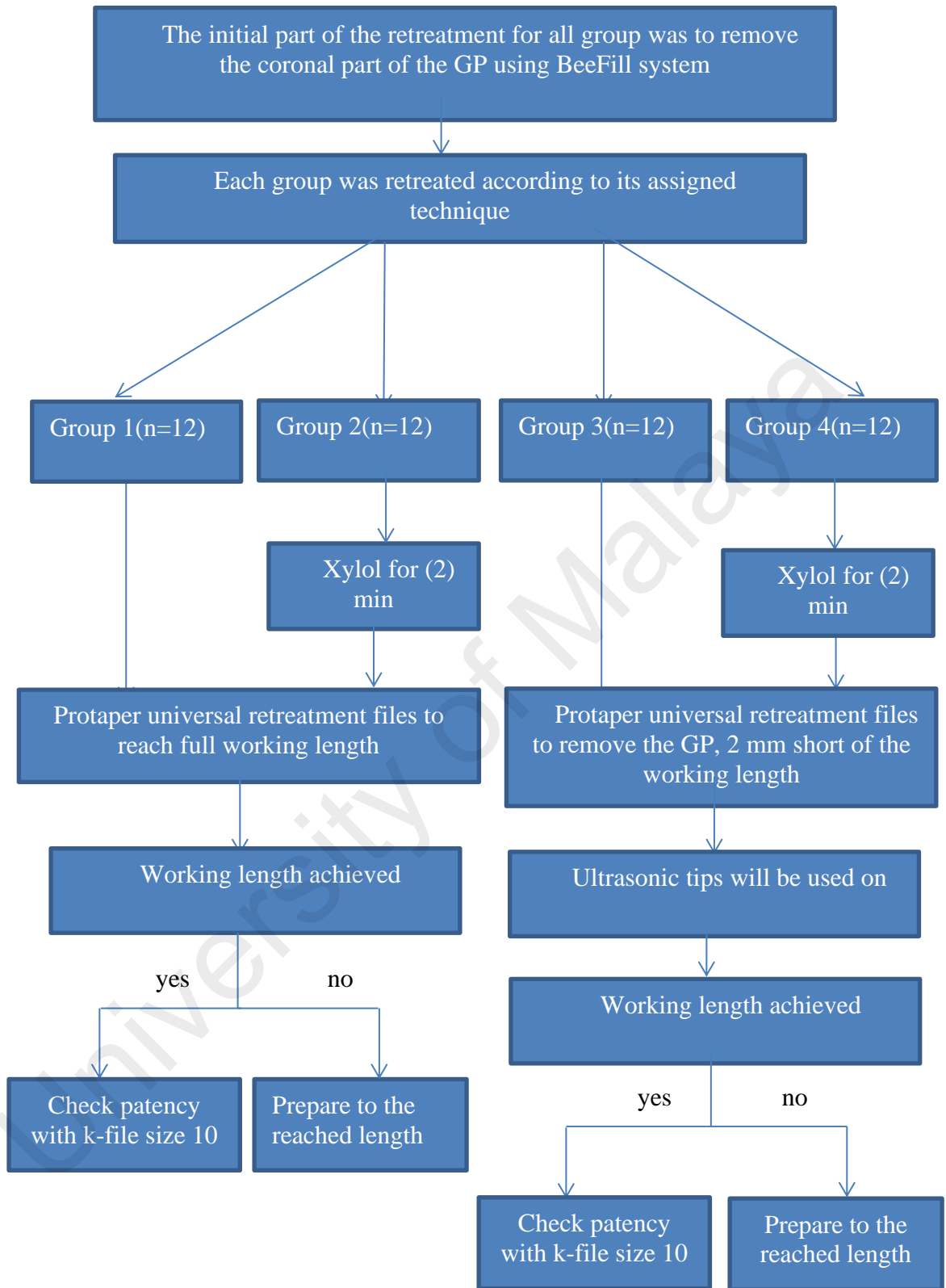


Figure 3.15 Retreatment procedure

CHAPTER 4: RESULTS

4.1 Comparison of the remaining root canal filling material volume between groups:

The remaining root canal filling material in the group used rotary files ($1.40 \times 10^5 \pm 1.97 \times 10^5 \mu\text{m}^3$) was the least among the groups while the group used rotary files and ultrasonic instrument ($3.78 \times 10^5 \pm 1.90 \times 10^4 \mu\text{m}^3$) was the highest. However, there is no significant difference found between the groups as shown in (Table 4.1).

Table 4.1 Comparison of the remaining root canal filling material between the groups

Group	1 (N=12)	2 (N=12)	3 (N=12)	4 (N=12)	P*
	Mean±(SD)	Mean±(SD)	Mean±(SD)	Mean±(SD)	
Volume of the remnant material (μm^3)	$1.40 \times 10^5 \pm (1.97 \times 10^5)$	$2.04 \times 10^6 \pm (1.24 \times 10^5)$	$3.78 \times 10^5 \pm (1.90 \times 10^4)$	$2.20 \times 10^6 \pm (1.20 \times 10^5)$	0.281

One-way ANOVA (significant at <0.05)*

Group 1= rotary files, group 2 = rotary files and Xylol, group 3 = rotary files and ultrasonic instrument, group 4 = rotary files, Xylol and ultrasonic instrument

4.2 Comparison of the percentage of the remaining root canal filling material between the groups:

The percentage of the remaining root canal filling material was the highest in the group used rotary files ($4.94 \pm 5.96\%$) while the group used rotary files and ultrasonic instrument had the least percentage ($1.73 \pm 0.80\%$). However, no significant difference was found between the groups as shown in (Table 4.2).

Table 4.2 Comparison of the percentage of the remaining root filling material between the groups

Group	1	2	3	4	P*
	(N=12)	(N=12)	(N=12)	(N=12)	
	Mean ±(SD)	Mean± (SD)	Mean± (SD)	Mean± (SD)	
Percentage of the filling material left	4.94± (5.96)	2.59± (4.16)	1.73± (0.80)	3.60± (3.20)	0.244

One-way ANOVA (significant at <0.05)*

Group 1= rotary files, group 2 = rotary files and Xylol, group 3 = rotary files and ultrasonic instrument, group 4 = rotary files, Xylol and ultrasonic instrument

4.3 Comparison of the percentage of the remaining root filling material in the coronal, middle and apical thirds

The repeated measure ANOVA showed a statistically significant difference between both the coronal (2.27±3.8%) and the middle (1.72±3.28%) thirds in comparison with the apical third (10.52±11.08%). In the comparison between the coronal and the middle third, there was no statistically significant difference found as shown in (Table 4.3).

Table 4.3 Comparison between the percentage of the remaining root filling material between the coronal, middle and apical thirds

	Coronal third	Middle third	Apical third	P*
	Mean ± SD	Mean ± SD	Mean ± SD	
percentage of the root filling left	2.27±3.8 ^a	1.72±3.28 ^b	10.52±11.08 ^{a, b}	<0.01*

Repeated measure ANOVA (significant at p<0.05)*

^a*Significant different between the coronal and apical third p<0.01*

^b*Significant different between the middle and apical third p<0.01*

4.4 Comparison of the volume of the remaining root canal filling material in the coronal, middle and apical thirds between groups

The one-way ANOVA test showed no statistical significance in the volume of the remaining root canal filling material in the coronal third of the root between the groups. The middle and apical thirds showed similar results as shown in (Table 4.4).

Table 4.4 Comparison of the remaining root canal filling material in the coronal, middle and apical thirds between groups

group	The volume of the Remaining material in root canal thirds			F (df)	P*
	Coronal	middle	Apical		
	Mean± SD	Mean± SD	Mean± SD		
1	35.42×10 ³ ± 50.40×10 ³	11.90×10 ³ ± 16.33×10 ³	18.46×10 ³ ± 19.68×10 ³		
2	55.59×10 ³ ± 14.24×10 ³	28.87×10 ³ ± 76.74×10 ³	40.29×1 ³ ± 56.18×10 ³	0.721 (102.37)	0.688
3	12.66×10 ³ ± 12.77×10 ³	10.44×10 ³ ± 17.20×10 ³	17.09×1 ³ ± 25.25×10 ³		
4	28.63×10 ³ ± 37.86×10 ³	67.04×10 ² ± 14.85×10 ³	25.99×10 ³ ± 33.11×10 ³		

One-way ANOVA (significant at p<0.05)*

Group 1= rotary files, group 2 = rotary files and Xylol, group 3 = rotary files and ultrasonic instrument, group 4 = rotary files, Xylol and ultrasonic instrument

4.5 Comparison of the percentage of remaining root canal filling material in the coronal, middle and apical thirds between groups

The one-way ANOVA test showed no statistical significance in the percentage of the remaining root canal filling material in the coronal third of the root between groups. The middle and apical thirds exhibited similar results as shown in (Table 4.5).

Table 4.5 Comparison the percentage of the remaining root canal filling material in the coronal, middle and apical thirds of the root between groups

Group	The remaining material in root canal thirds (%)			F (df)	P*
	Coronal	middle	Apical		
	Mean ± SD	Mean ± SD	Mean ± SD		
1	2.31 ± 2.86	1.77 ± 2.11	7.94 ± 7.13	0.944(102.37)	0.491
2	3.49 ± 6.61	2.52 ± 5.63	13.11 ± 15.63		
3	1.05 ± 1.21	1.62 ± 2.27	7.40 ± 4.91		
4	2.23 ± 2.29	0.95 ± 1.78	13.62 ± 13.03		

One-way ANOVA (significant at $p < 0.05$)*

Group 1 = rotary files, group 2 = rotary files and Xylol, group 3 = rotary files and ultrasonic instrument, group 4 = rotary files, Xylol and ultrasonic instrument

4.6 Comparison of re-establishing patency between the groups:

The overall percentage of re-establishing patency was 89.6%. Among groups, the highest percentage of re-establishing patency was achieved in the group used rotary files and ultrasonic instruments (100%) while lowest was in the group that used rotary files only and the group used rotary files, Xylol, and ultrasonic instruments. No statistical significance was found between the groups as shown in (Table 4.6).

Table 4.6 Comparison of re-establishing patency between groups

Group	N	Percentage of the sample achieved patency(N)	P*
1	12	83.3% (10)	0.483
2	12	91.7% (11)	
3	12	100% (12)	
4	12	83.3% (10)	
Overall	48	89.6% (43)	

Chi-square test (significant at $p < 0.05$)*

Group 1 = rotary files, group 2 = rotary files and Xylol, group 3 = rotary files and ultrasonic instrument, group 4 = rotary files, Xylol and ultrasonic instrument

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CHAPTER 5: DISCUSSION

5.1 Selection of teeth

Extracted human teeth were chosen in this study in an attempt to simulate the clinical situation. However, it is known that human teeth have substantial variation in root morphology. Hence, multiple measures have been taken in order to ensure minimal variation between the groups. mandibular premolars with straight roots were used in this study as these teeth usually have one straight canal and they are easy to obtain as they are frequently extracted for orthodontic purposes. In order to accurately measure the efficiency of removing root canal filling material, studies tried to standardize the sample (Agrafioti et al., 2015; Donnermeyer, Bunne, et al., 2018; Uzunoglu et al., 2015). In this study, the sample was standardized according to the following: canals with similar shape (long oval), canals with curvature less than 20° in both buccolingual and mesiodistal radiographic views, and canals with the apical size of K-file size 15 or less. After that, the teeth were decoronated at the level of 13 mm from the apex. Although decoronation does not simulate the clinical situation, standardization of the sample not only facilitates working but also eliminates any interferences by the crowns during initial treatment and subsequent retreatment.

5.2 Preparation of the canals

The canals were prepared using the crown-down technique. The advantages of these techniques were listed by Goerig et al. (1982) as the following:

- 1- It provides straighter line access to the apical area.
- 2- It eliminates the coronal two-thirds interferences which allow quick and efficient apical instrumentation.
- 3- It reduces the apically extruded debris by removing the bulk of the pulp tissue, debris, and micro-organisms before apical instrumentation.

- 4- It allows deeper irrigation penetration through radicular access enlargement.
- 5- It establishes the working length throughout the preparation stages.

The canal morphology of mandibular premolars have been reported to be oval in more than 56% in human dentition (Wu et al., 2000). The task of shaping oval canals was reported to be challenging leaving behind untouched areas or recess buccally and/or lingually (De-Deus et al., 2011; Siqueira Jr et al., 2010). Most of the available rotary and reciprocating systems failed to ideally prepare oval canal systems leaving unprepared walls (Arias et al., 2018). To overcome the problem of having underprepared dentine, Self-Adjusting File (SAF) was introduced as a single instrument able to adapt itself in 3-dimensions to the irregularities of the root canal system. However, Versiani et al. (2013) reported similar results in the efficiency of preparing oval root canal systems between SAF and other rotary and reciprocating systems (Versiani et al., 2013). It was suggested to use conventional K-hand instruments in circumferential filling to prepare the untouched recess during cleaning and shaping. However, Weiger et al. (2002) reported similar results in circumferential filling in both manual and rotary files in the preparation of oval canals (Weiger et al., 2002). Thus, in this study circumferential filling using rotary instruments was used.

Clinically, sufficient cleaning and shaping cannot be predictably confirmed so as to determine the shaping endpoint. Siqueira et al. (2019) listed the suggested criteria as the following:

- 1- Dentinal filings collected on instruments are clean after visual inspection.
- 2- The irrigating solution is clean when collected in a gauze after rinsing the canal.
- 3- The clinician has a tactile feeling of glass smooth walls.
- 4- Preparation size is determined based on mean apical diameters for the different groups of teeth.

5- The canal is enlarged two to four sizes beyond the first file to bind.

Unfortunately, none of the previously mentioned guidelines is accurate. Even canals fulfilling all of the previously mentioned criteria might still have tissue remnants (Siqueira et al., 2019). Studies reported that the larger the apical preparation size, the greater the depletion of bacteria in the root canal system (Mickel et al., 2007; Siqueira Jr et al., 1999). However, a prospective study compared the apical preparation size in relation to the first bind file reported no additional benefit in canals prepared more than three sizes larger than the first binding file (Saini et al., 2012).

Irrigation is considered as the most important part in root canal treatment, especially in the extermination of bacteria from the root canal system (Haapasalo et al., 2014). Irrigation helps in killing and removal of the microorganisms, necrotic and inflamed tissue and dentinal debris (Haapasalo et al., 2010). Furthermore, irrigation solutions dissolve tissues, lower the friction between the files and dentinal walls, increase the cutting efficiency of the files and reduce the temperature of the tooth especially upon ultrasonic activation (Haapasalo et al., 2014). Irrigation solutions also may prevent preparation mud from packing in the apical end of the root canal (Park et al., 2012). In order to improve the irrigation regime, small size gauge needle preferably 30-gauge should be used to gain access to the apical end of the root canal system (Park et al., 2013). Also, it has been reported that passive ultrasonic activation of irrigation solution is an adjunctive treatment for cleaning and shaping and it is considered more effective than syringe irrigation (van der Sluis et al., 2007).

In this study, canals were prepared to size 30 K-file (X3, Protaper NEXT®) which is three sizes larger than the first binding file. Needle 30-gauge was used to ensure delivery of irrigation solutions to the apical end of the root canal system in conjugation with ultrasonic passive activation.

5.3 Root canal filling material (iRoot SP)

Calcium silicate based sealers consist of two groups sharing a common setting reaction. The first group (1- component) is a premixed calcium silicate based sealer which requires an external water supply to set, while the second group (2-component) is a calcium silicate based sealer which required an internal water supply to set, iRoot SP belongs to the first group. Varying results were reported regarding iRoot SP setting time. The setting times ranged from 2.7h (Zhou et al., 2013) up to 240h (Loushine et al., 2011). One study reported that BC sealer did not completely set in 4 weeks (Lee et al., 2017). However, in their experiment, they used a stainless steel ring on a glass slab which might be the reason for the BC sealer as it will now allow water to reach the sealer. In this study, obturated canals with iRoot SP were incubated in a (37°C,100% relative humidity) for two weeks which is four days more than the highest reported number of 240h to make sure the sealer is fully set. This approach has been adopted by several studies (Agrafioti et al., 2015; Hess et al., 2011; Uzunoglu et al., 2015).

5.4 Root canal filling material removal

In the present study, Xylol was used as it was reported to be the most effective among solvents (Martos et al., 2011; Tanomaru-Filho et al., 2010). The application of Xylol for 5 minutes was reported to be the most effective (Martos et al., 2011). The coronal 3 mm of the root canal filling was removed in order to serve as a reservoir for the solvent and facilitate the access for further re-treatment files (Friedman et al., 1990).

ProTaper® universal re-treatment files were used according to the manufacturer instruction regarding the speed, torque control and protocol of use. The use of rotary instruments proved to be a time saver (Giuliani et al., 2008; M Hülsmann & V Bluhm, 2004), safe (Rödig et al., 2014), and efficient in the removal of root canal filling material (Ersev et al., 2012; Gu et al., 2008).

Ultrasonic instruments have been used with light pressure on the walls of the canals in order to pulverize the cement and then with copious irrigation, the debris will move coronally out of the canal system (de Mello Junior et al., 2009; Friedman et al., 1990). Furthermore, ultrasonic agitation of irrigation solution during root canal retreatment procedure was reported to be beneficial in the removal of root canal filling material (Bernardes et al., 2016). For the previously mentioned reasons, ultrasonic instruments were used in this study.

Studies reported enhanced removal of the remaining root canal filling material when the apical size was enlarged by one or two sizes beyond the original apical size before the retreatment procedure (Ezzie et al., 2006; Hassanloo et al., 2007; Schirrmeister et al., 2006). This protocol was adopted by multiple studies which evaluated the removal of bioceramic sealer (Hess et al., 2011; Oltra et al., 2017; Uzunoglu et al., 2015). The canals in this study were enlarged by two sizes beyond their original size before retreatment to size 40 k-file (X4 Protaper NEXT®).

5.5 Remnant material evaluation method

The remaining root filling material was evaluated using various techniques such as digital radiography (Ersev et al., 2012), scanning electron microscopy (Hess et al., 2011), confocal microscopy (Kim et al., 2015), micro CT (Suk et al., 2017) and optical microscopy (Donnermeyer, Bunne, et al., 2018) . The radiographs and digital images of sectioned teeth offer two-dimensional information about a three-dimensional structure. Hence, no accurate measurement of the total canal area is feasible. Furthermore, sectioning the teeth might cause loss and dislocation of the filling remnants. Moreover, the subjective evaluation of the remaining material between observers is another limitation of this technique (Uzunoglu et al., 2015). Scanning electron microscopy can qualitatively assess the cleanliness of dentinal tubules (Simsek et al., 2014). Micro-CT is non-invasive

and reproducible evaluation methods that can quantify the remaining debris (Zuolo et al., 2016). However, it can't distinguish the proportion of gutta-percha and sealer remnants (Ma et al., 2012). Most studies on the removal of root filling material used a voxel size between 17 μm and 30 μm . Yet some studies used as high resolution as 3 μm (Asheibi et al., 2014). However, there was no mention if there is any implication of using such high resolution on the results. In fact, it was suggested that a voxel size between (34 μm - 68 μm) to be sufficient for endodontic studies (Peters et al., 2000)

Micro-CT scanning was used in this study for the advantages mentioned above with a Voxel size of 18 μm . As the objective of this study is to remove the entire filling material regardless of whether it was gutta-percha or sealer, the limitation of micro-CT assessment technique will not affect the outcome of this study.

5.6 Remaining root canal filling material

Intra-radicular infection caused by newly developed or re-established bacterial biofilm after root canal treatment is the major cause for primary root canal treatment failure (Nair, 2004a). Thus, the outcome of non-surgical root canal treatment depends on proper cleaning and shaping of root canal system including the previously untouched areas (Khalilak et al., 2013). To ensure adequate disinfection of the root canal system is possible, complete removal of present root canal filling material is a pre-requisite (Zehnder & Paque, 2008). Recently, calcium silicate based endodontic sealers have been introduced with good antimicrobial activity (Wang et al., 2014) and excellent biocompatibility (Camps et al., 2015). However, doubts have raised regarding the removal of calcium silicate based sealer due to its interaction with dentine surface (Atmeh et al., 2012) and bonding to dentine (Kaup et al., 2015).

A substantial amount of studies reported that regardless of the removal technique of the root canal filling and instruments used, residues of the root filling material were found

in the root canal system (Ma et al., 2012; Uzunoglu et al., 2015; Zuolo et al., 2016). These findings come in agreement with the results of this study. None of the used techniques was able to remove 100% of the root filling material.

The remnant of the root filling material in the coronal, middle and apical thirds of the root system was also reported. The vast majority of the studies reported a higher percentile of the root filling remnant in the apical third (Crozeta et al., 2016; Ersev et al., 2012; Kontogiannis et al., 2019; Ma et al., 2012; Simsek et al., 2014; Uzunoglu et al., 2015), consistent to the present study. This finding can be explained by the difficulty to clean and shape the apical third of the root compared to the coronal and middle thirds (Yu & Schilder, 2001). On the other hand, Donnermeyer, Bunne, et al. (2018) reported no significant difference in the percentage of the remaining root filling material between the thirds of the root canal (Donnermeyer, Bunne, et al., 2018). This might be explained by the methodology used in their study as they sectioned the sample then it was examined under a light microscope and the percentage of the remaining material was calculated using ImageJ software. The sectioning of the sample might have caused loss and dislocation the root filling material and the use of 2d images to calculate the volume both might have affected the results.

5.7 Re-establishing working length and patency

The ability to reach the working length and patency was investigated by most of the studies. Hess et al. (2011) compared the retreatability of Endosequence BC sealer and AH Plus with gutta-percha cone placed to 2 mm short of the WL. The WL was acquired in 30% of the sample obturated with Endosequence BC sealer and gutta-percha cone 2 mm short of the WL. This result was significantly lower compared to the other groups (Hess et al., 2011). On the contrary, Agrafioti et al. (2015) conducted a study comparing TotalFill BC sealer, AH Plus and MTA Fillapex using the same procedure described

above. Results showed WL achievement in all the samples (Agrafioti et al., 2015). Agrafioti et al. (2015) explained the difference in the results by more the more complex anatomy of the mesiobuccal canals of mandibular molars. However, it important to mention that Agrafioti et al. used warm vertical compaction with BC Sealer. Hence, using warm vertical compaction can deteriorate the physical properties of bioceramic sealer (Qu et al., 2016). It might be an important determining factor behind their results. In the studies with no apical plug Oltra et al. (2017) reported a 93% and Zuolo et al. (2016) reported an 85% success rate of WL recovery in the groups filled with Endosequence BC sealer (Oltra et al., 2017; Zuolo et al., 2016). Two studies reported that patency was achieved in all the samples filled with bioceramic sealer and gutta-percha (Donnermeyer, Bunne, et al., 2018; Kim et al., 2015). This study had an 89.5% success rate in achieving working length and patency which is similar to the majority of the previous studies. The difference with that of Hess et al. (2011) might be explained by the more complex anatomy of the mesiobuccal canals of mandibular molars used in their study.

5.8 Limitations of the study

This study was performed on straight long oval canals. Other root canal morphological variations such as curved canals or single roots with multiple canals with or without isthmus were not studied. In addition to that the teeth were decoronated which doesn't resemble the clinical situation.

CHAPTER 6: CONCLUSION AND SUGGESTIONS

6.1 Conclusions

The efficiency of four different techniques in the removal of bioceramic sealer iRoot SP[®] from long-oval root was examined. With the limitations of this study, the following conclusions were attained:

1. No technique proved to be superior to the others in removing bio-ceramic root filling-based material.
2. The apical third retained a significantly higher percentage of its filling material compared to the middle and coronal third.
3. Bio-ceramic sealers are negotiable in straight, single long oval root canal anatomy.

6.2 Clinical implications

1. All techniques can be used successfully in removing the bioceramic sealer. However, none of the techniques completely removed the root filling material
2. Patency can be predictably achieved in canals obturated with bio-ceramic sealer.

6.3 Recommendations for futures studies

1. Investigation on different canal morphologies
2. The use of different types of rotary or reciprocating file systems, ultrasonic instruments, and solvents.
3. The use of different obturation techniques as a new warm vertical compaction friendly bioceramic sealer has been introduced.
4. The use of microscope in the retreatment procedure.

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